LIPOSOMAL DRUG DELIVERY OF ZIDOVUDINE AND IT'S EVALUATION

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Dedicated to -
My Loving Parents

INSTITUTE OF PHARMACY
BUNDELKHAND UNIVERSITY, JHANSI

Certificate

This is to certify that the thesis entitled, "**Liposomal Drug Delivery of Zidovudine and its Evaluation**" submitted to the Bundelkhand University, Jhansi (U.P.), in fulfillment of requirements for the award of degree of **Doctor of Philosophy** in **Pharmacy** embodies the original research work carried out by **Mr. S. PALANI** under my supervision and has not been submitted in part or full for any other degree or diploma of this or any other University. It is further certified that he has devoted more than 24 months i.e. the minimum stipulated period, for completion of the work.

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Declaration

The work entitled, "**Liposomal Drug Delivery of Zidovudine and its Evaluation**" embodies the results of the original research work carried out by me in the **Institute of Pharmacy**, Faculty of Pharmacy, Bundelkhand University, Jhansi. This work has not been submitted in part or full for any other degree or diploma of this or any other University.

Dated: 18/09/07

Place: Jhansi

18/09/07

Mr. S. PALANI

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Chapter- 1

Introduction

Chapter-1

INTRODUCTION

Prof. A.D Bangham of the United Kingdom demonstrated the preparation of liposomes, with entrapped solutes, for the first time in 1965. The idea of using liposomes for drug delivery has been around since the early 1970s. Liposomes are microscopic spheres made from fatty materials, predominantly phospholipids. Because of their similarity to phospholipid domains of cell membranes and an ability to carry drug substances, liposomes can be used to protect active ingredients and to provide time-release properties in medical treatment. Liposomes are made of molecules with hydrophilic and hydrophobic ends that form hollow spheres. They can encapsulate water-soluble ingredients in their inner water space, and oil-soluble ingredients in their phospholipid membranes. Liposomes are made up of one or more concentric lipid bilayers, and range in size from 50 nanometers to several micrometers in diameter.

The goals are to protect the body from unwanted side effects of various drugs and when made to be targeted to specific tissue, to achieve desired concentrations of these drugs at a target site. Despite much research, one hurdle has been to find ways to prevent the body from breaking down liposomes while they are still in the bloodstream and before they reach a site. Conventional liposomes are limited in effectiveness because of their rapid uptake by macrophage cells of the immune system, predominantly in the liver and spleen. With regard to the short in vivo half-life of conventional liposomes, Researchers at a number of industries have overcome this obstacle by designing liposomes that are nonreactive, sterically stabilized (by using polymers) attached to prevent the liposomes from sticking to each other and to blood cells or vascular walls. 'Stealth' liposomes appear to be invisible to the immune system and have shown encouraging results in therapy. Thus, coating liposomal vesicles with hydrophilic polymers reduces uptake by the liver. As a result, coated liposomes remain in circulation longer than conventional liposomes. Also, by incorporating targeting ligands on the surface of the liposomes, it is possible to direct them to certain organs.

1.1 AIM OF THE WORK:

The proposed research is concerned with the study of the role of polymers in controlling the interfacial interactions of liposomes, drug transport kinetics across the liposomal gels and the quantification of stabilization introduced by polymers. The aim of this study is to investigate the effects of experimental conditions on the interaction of liposomes at the solid-liquid interface, drug release from conventional (at different conditions like temperature, pH, and salt) and polymer-coated liposomes, as well as their physical and chemical stability.

The research work is focused on the following studies:

- To study the interactions between the drug and the polymer selected for the formulation
- Formulation of Conventional liposomes, Liposomal Gels
- To study the effect of the different ratios of PC and Cholesterol on the formulation
- To study the Impact of stirring speed and hydration time on the formulation of liposomes
- To characterize the formulated liposomes
- To study the morphology of the liposomes
- To study the effect of temperature, pH and salt on the liposomal size
- Analysis of the formulated liposomes by DSC
- To determine the particle size of the formulated liposomes
- To determine the zeta potential of the formulated liposomes
- To determine the percentage drug entrapment with in the formulated liposomes
- To study the effect of ratios of PC and cholesterol on drug entrapment
- Invitro release analysis of the formulated liposomes
- To study the effect of temperature, pH, Stirring speed, Particle size,
- Salt concentration on the drug release from formulated liposomes
- Stability analysis of the formulated liposomes
- Invivo testing of the liposomal gel on rabbit model

Chapter-2

Literature

Chapter-2

LITERATURE

2.1. LIPOSOMES :

Liposomes are vesicles containing lipid bilayers surrounding aqueous compartments, whose diameters can vary from 20 nm to 1 μm. They have been used for more than 30 years as vehicles to improve the delivery of various drugs, such as anticancer drugs (doxorubicin), antibiotics (anthracycline, amphotericin B) or vaccines[1].

Lipophilic molecules can be incorporated into the lipid bilayer of liposomes, while hydrophilic components can be entrapped in the inner aqueous phase. Liposomes have been used as drug delivery system in various therapeutic applications such as gene therapy, drug targeting and many others, mainly because of the following advantages[2].

1. They are biodegradable and non-toxic.
2. Most molecules can be incorporated into liposomes without modification at a very high concentration.
3. Biologically active molecules are protected by the lipid bilayer of liposome from damage by chemicals and enzymes after injection into the blood stream of an organism.
4. The host cells are also protected from the toxicity of the entrapped molecules.

During these last 30 years, different liposomes have been developed in order to improve their stability in vivo. Conventional liposomes are the original ones, composed of phospholipids and cholesterol and are stable enough for *in vitro* experiments, but in vivo they are rapidly taken up either by the organs of the reticuloendothelial system such as the liver and spleen or by phagocytic cells such as macrophages. To prolong the liposome half- life in the circulation, liposomes are coated with polyethylengycol (PEG), reducing interactions with plasma proteins or receptors and these liposomes are called "stealth liposomes". The most recently developed type of liposomes is the cationic liposomes, used for the gene therapy.

Their cationic lipids can interact with a negatively charged DNA to form a neutral noncovalent complex.

The major component of liposomes is phosphatidylcholine (PC), which can be extracted from natural sources such as egg yolk, brain tissues or Soya beans or can be prepared synthetically[3]. Charged lipids such as Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), Dordioleoyltrimethylammonium propane (DOTAP) are added to introduce charges. Cholesterol is ordinarily included to further improve mechanical stability and to decrease leakage of the encapsulated contents. To prevent the oxidation of the lipids, antioxidants such as a-tocopherol or ascorbic acid are often added [4].

2.2 DRUG DELIVERY :

Humankind's efforts to confront disease dates back to early civilization. Substances taken from nature were tested and used to treat dysfunctions of physiological life processes, pain and discomfort. With the advancement of science, the active ingredients of these materials, the drugs, were identified, isolated and in many cases their mechanism of action elucidated. New drug candidates are tested even today in the quest to add increasingly effective tools against diseases. Drug characteristics differ dramatically, even those aimed to treat the same symptoms; chemical composition, size, hydrophilicity and potency identify molecules whose function may be specific or highly complex. An increasing understanding of cellular biology at the molecular level, combined with the (decoding) of the human genome, and a technological breakthrough in the field of proteomics and DNA micro-arrays, has introduced even more applicants, like peptides and nucleic acids (gene delivery). Drug activity is a result of molecular interaction(s) in certain cells; it is therefore easily deduced that it is necessary for the drug to reach somehow the site of action following administration (oral, intravenous, local, transdermal, etc.) at sufficient concentrations. The scientific field dealing with this issue is known as drug delivery and has essentially the following aim: to deliver the drug at the right place, at the right concentration for the right period of time. When this is impossible by simply selecting an appropriate administration route, or if such administration causes patient discomfort, strategies based on the association of the drug with a carrier are an

alternative. Additional motivations for such approaches include the reduction of required resources for therapy, accomplished by an increase of the drug's therapeutic index and the prevention of frequent, unpleasant or expensive treatments.

Drug delivery systems, ranging from implantable electronic devices to single polymer chains, are required to be compatible with processes in the body (biocompatibility) as well as with the drug to be delivered. Drug delivery system alter the biodistribution and pharmacokinetics of the associated drug: that is the time-dependent percentage of the administered dose in the different organs of the body. Furthermore, obstacles arising from low drug solubility, degradation (environmental or enzymatic), fast clearance rates, non-specific toxicity, inability to cross biological barriers, just to mention a few, may be addressed by DDS2. Overall, the challenge of increasing the therapeutic effect of drugs, with a concurrent minimization of side effects, can be tackled through proper design and engineering of the DDS, in a case-to-case manner.

2.3 LIPOSOMES AS DRUG-DELIVERY VEHICLES :

The use of liposomes as vehicles for selective delivery of drugs to specific tissues has received considerable attentions[5]. By virtue of their biodegradable and nontoxic nature, liposomes can be safely administered without severe side effects. The first results were rather disappointing because the first generation of liposomes referred to as conventional liposomes (C-liposomes) were unstable in biological fluids and inefficient in drug loading. For drug delivery, liposomes can be administrated topically or parenterally. After systemic (usually intravenous) administration, which seems to be the most promising route for this carrier system, liposomes are recognized as foreign particles and are taken up by the cells of the mononuclear phagocytic system (MPS), mostly Kupffer cells in the liver and macrophages of the spleen. In addition, C-liposomes are highly unstable in biological fluids, leading to a rapid release of encapsulated molecules mainly due to the interactions with two distinct groups of plasma proteins, HDL and opsonins adsorbed onto liposome surface and mediating their endocytosis by MPS. Therefore, the rate of liposome clearance from blood circulation depends on the ability of opsonins to bind to the

liposome surface; nonetheless this can be manipulated through the appropriate selection of liposome characteristics. In addition, the clearance of liposomes from blood stream depends on the liposome properties such as bilayer fluidity, surface charge and vesicle size.

The pronounced tendency of C-liposomes to be taken up that is to target cells of MPS is very useful for delivering drugs to macrophages but restrain the in vivo use of liposomes for selective delivery of drugs to other sites. Thus, the C-liposome uptake by MPS cells has limited the development of liposomes as drug delivery systems for over 20 years. After numerous and various studies, new formulations of liposomes with increased stability were designed; thus, liposomes that contain lipidic derivatives of polyethylene-glycol (PEG) which possess the properties to avoid MPS uptake and show increased times in blood circulation were found particularly appropriate[6]. Also, "smart" liposomes that can tolerate specifically induced modifications of the bilayers or can be covered with different molecules were constructed. These sort of liposomes include proteoliposomes containing fusogenic proteins[7], pH-sensitive liposomes (able to avoid lysosomal degradation[8], cationic liposomes (form complexes with DNA[9,10], target sensitive liposomes (disintegrate after binding to a target cell and release the content in the cell vicinity and immunoliposomes (directed toward specific sites by coupling antibodies to their surface[11].

At present, researchers in the liposome field are trying to reach the concept of "magic bullet" introduced by Paul Ehrich in 1906. Thus, "smart" liposomes capable to deliver specifically drugs or genes to a certain cell or tissue have been designed; still, it remains to be validated by in vivo and clinical studies. After numerous and various studies, new formulations of liposomes with increased stability were designed; thus, liposomes that contain lipidic derivatives of polyethylene-glycol (PEG) which possess the properties to avoid MPS uptake and show increased times in blood circulation were found particularly appropriate. Also, "smart" liposomes that can tolerate specifically induced modifications of the bilayers or can be covered with different molecules were constructed. These sort of liposomes include proteoliposomes containing fusogenic proteins[12], pH-sensitive liposomes (able to avoid lysosomal

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2.4 LIPOSOMES:

Liposomes are vesicles containing lipid bilayers surrounding aqueous compartments, whose diameters can vary from 20 nm to 1 μm. They have been used for more than 30 years as vehicles to improve the delivery of various drugs, such as anticancer drugs (doxorubicin), antibiotics (anthracycline, amphotericin B) or vaccines).

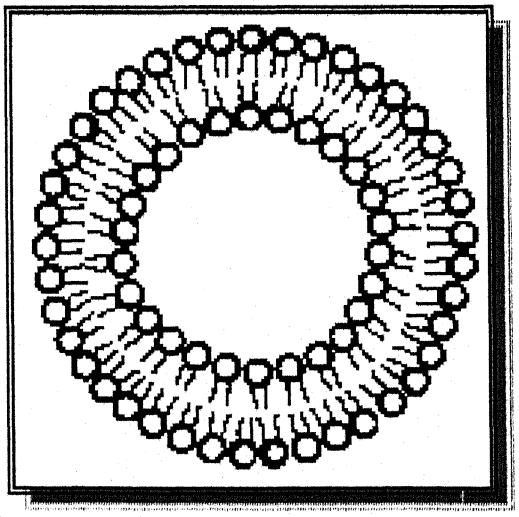

Figure-1: Liposome showing a single phospholipid bilayer (Unilamellar Liposomes)

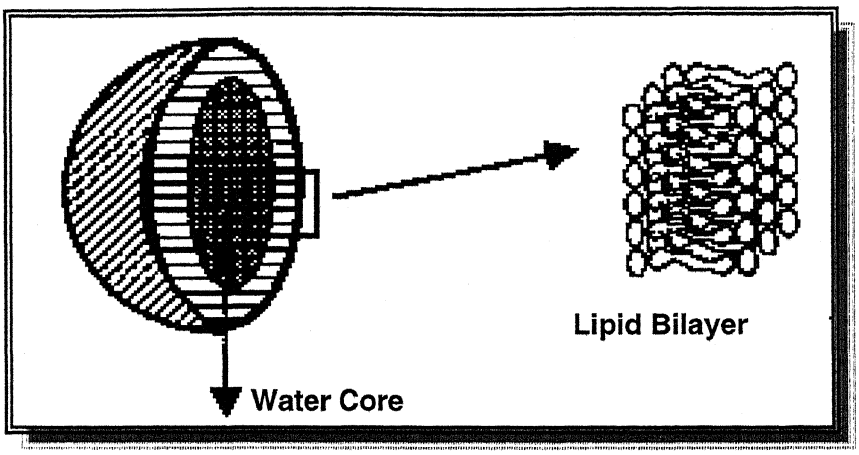

Figure-2: Schematic view of liposomes.

Lipophilic molecules can be incorporated into the lipid bilayer of liposomes, while hydrophilic components can be entrapped in the inner aqueous phase as shown in Figure-2. Liposomes have been used as drug delivery system in various therapeutic applications such as gene therapy, drug targeting and many others, mainly because of the following advantages:

1. They are biodegradable and non-toxic.
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developed type of liposomes are the cationic liposomes, used for the gene therapy. Their cationic lipids can interact with a negatively charged DNA to form a neutral noncovalent complex.

The major component of liposomes is phosphatidylcholine (PC), which can be extracted from natural sources such as egg yolk, brain tissues or Soya beans or can be prepared synthetically[3]. Charged lipids such as phosphatidylglycerol (PG), phosphatidylethanolamine (PE) or dioleoyltrimethyl-ammonium propane (DOTAP) are added to introduce charges. Cholesterol is ordinarily included to further improve mechanical stability and to decrease leakage of the encapsulated contents. To prevent the oxidation of the lipids, antioxidants such as a-tocopherol or ascorbic acid are often added[4].

2.5 PHYSICOCHEMICAL PROPERTIES OF LIPOSOMES :

The physicochemical properties of the liposome drug product are critical to ensuring drug product quality. Therefore, a detailed evaluation of these properties should be provided. Rigorous characterization of the physicochemical properties can also be beneficial in evaluating subsequent changes in manufacturing. The physicochemical characterization tests, which are critical to ensuring product quality of each batch of liposome drug product, should be identified. However, all the characterization tests need not be included in the specifications. Properties specific to liposome drug products that may be useful to assess include:

- Morphology of the liposome, including lamellarity determination, if applicable
- Net charge
- Volume of entrapment in liposomal vesicles
- Particle size (mean and distribution profile)
- Phase transition temperature
- Spectroscopic data, as applicable
- *In-vitro* release of the drug substance from the liposome drug product

- Osmotic properties
- Light scattering index

The morphology study of liposomes shows

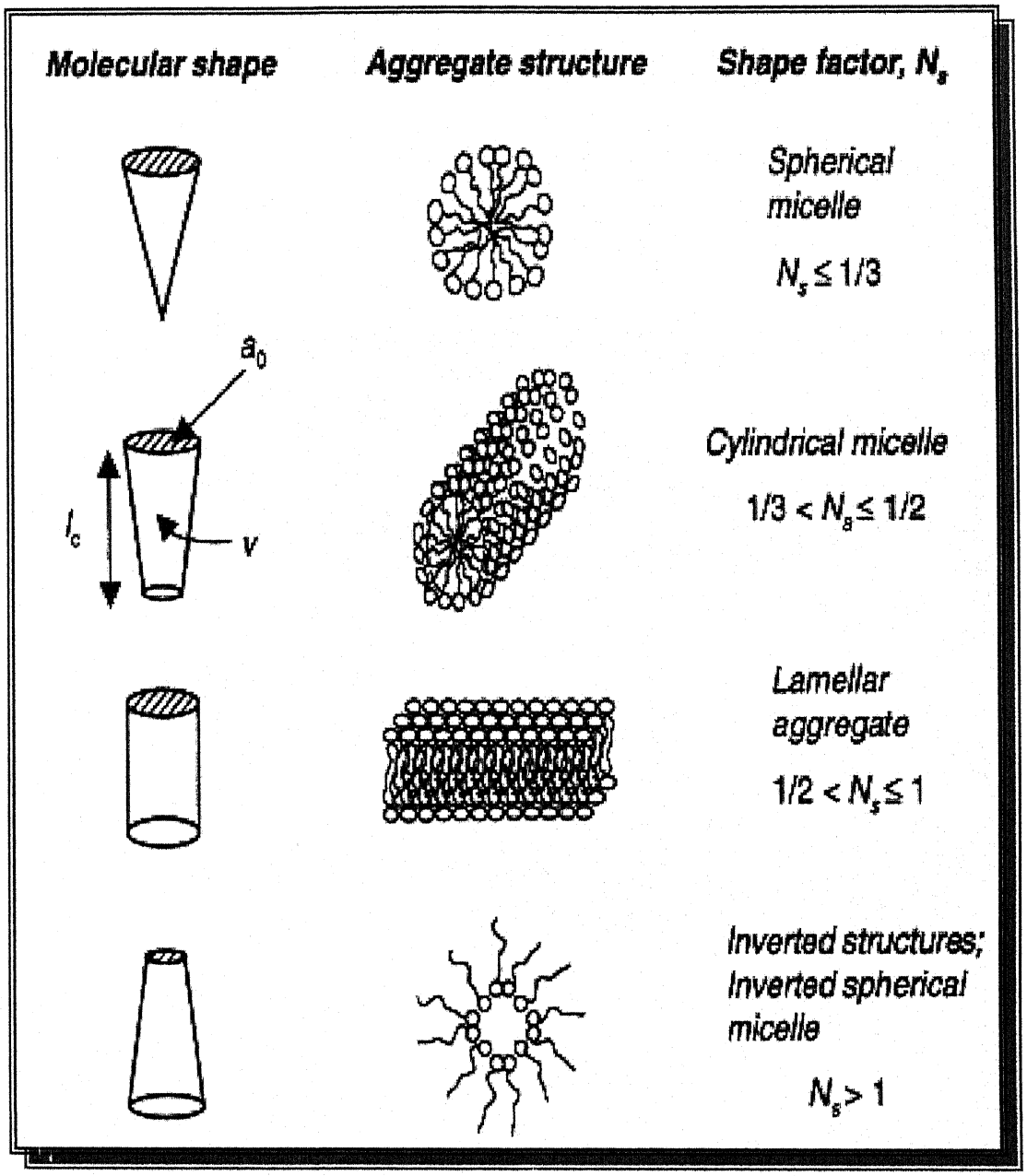


Figure-3: Predicted aggregate structure as a function of the factor.

Further the study of the liposomes reveals the organization of PC in aqueous medium

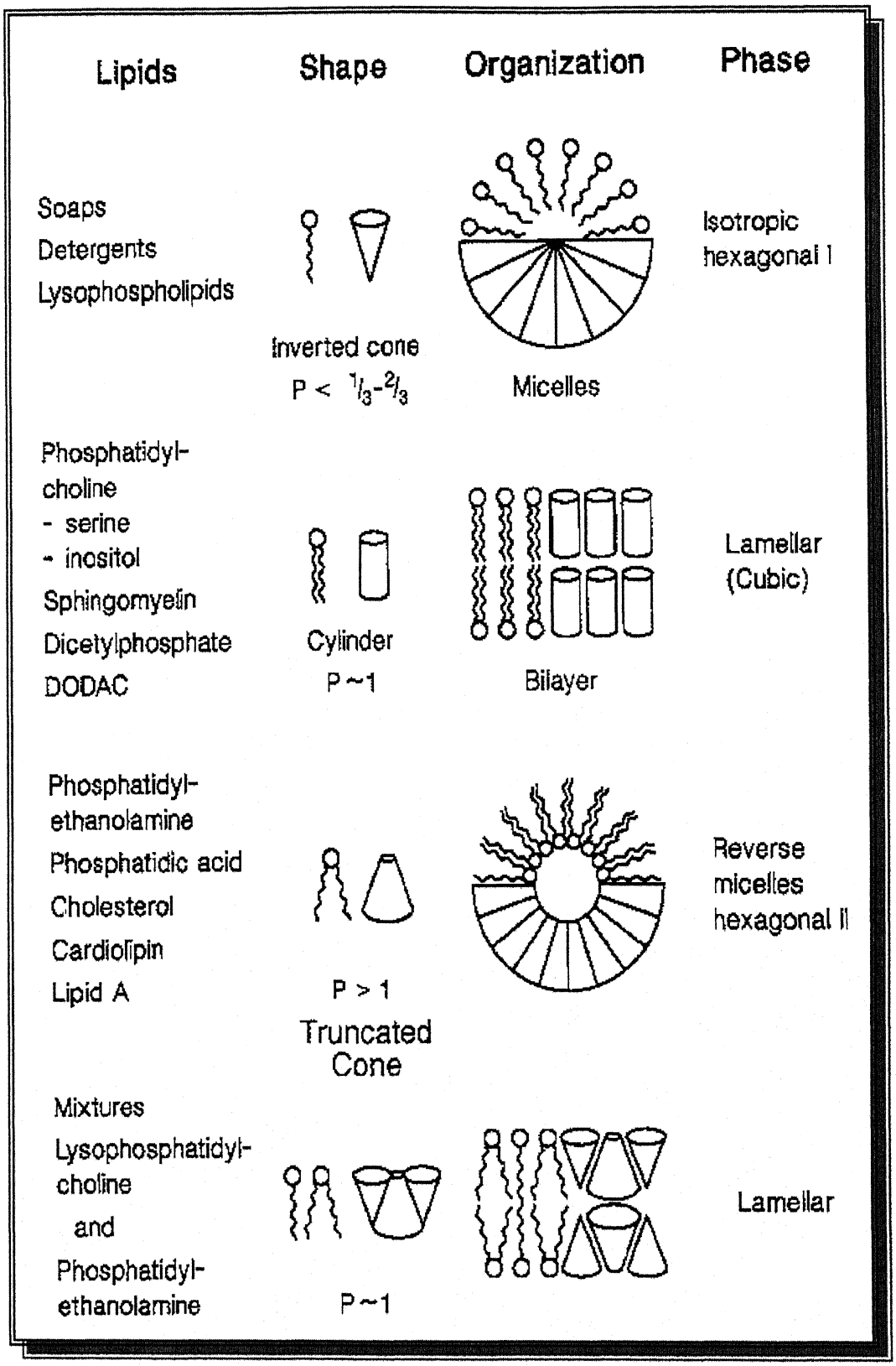


Figure- 4: Different shape and organization of Phosphotidylcholine in aqueous medium

2.6 LIPOSOMES COMPOSITION:

Liposomes are composed of neutral or anionic lipids, which can be extracted from natural sources or prepared synthetically. The most used natural lipids are lecithins (phosphotidylcholines), Sphingomyliens, and phosphotidylethanolamines which are normally extracted from natural sources, such as egg yolks, Soya beans and brains. These lipids are physiologically neutral. Negatively charged lipids are phosphotidylserines, phosphotidylglycerols. Natural lipids contain various mixtures of lipid chains. Synthetic lipids have well defined acylchains attached to the polar ends. The lipids are dimrystoyl, dipalmityl, dioleoyl. Cholesterol is often added to improve mechanical stability of the bilayer and decrease leakage of the encapsulated drug materials.

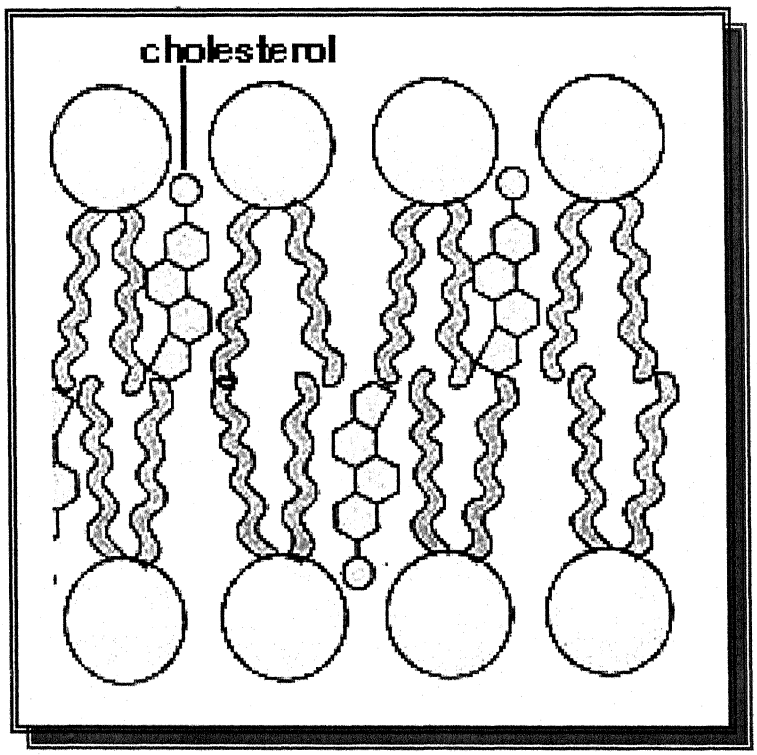


Figure-5: Cholesterol arranged between phospholipid bilayer

Role of cholesterol in vesicle Rigidization:

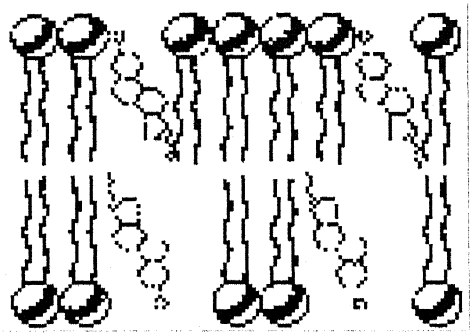
Cholesterol is known to have important modularity effect on the bilayer membrane[14-15]. Cholesterol acts a "fluidity buffer', since below the phase transition it tends to make the membrane less ordered while above the transition it tends to make the membrane more ordered, thus suppressing the tilts and shift in membrane structure specifically at the phase transition. Though cholesterol it self does not form

bilayers, but it can be incorporated into phospholipids membrane in very high concentrations upto 1:1 or even 2:1 molar ratio of cholesterol to PC.

Being an amphipathic molecule, cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface, and the aliphatic chain aligned parallel to the acyl chains in the centre of the bilayer. Above a certain concentration of cholesterol, the membrane area occupied by combination of acyl chains and cholesterol is greater than (or equal to) that taken by phosphocholine head-group. This could be the possible mechanism for phospholipid membrane with high level of cholesterol that retard the chain tilt. The tilt is observed in the gel phase of liposome composed of pure PC to maximize lipid chain interaction. Addition of cholesterol to PC membranes at lower concentration has a marginal effect in the transition temperature, but with increased concentration (~50 mole% cholesterol) it eliminates evidence of a phase transition at original to altogether by reducing the enthalpy of phase change to zero value. In doing so, it alters the fluidity of the membrane both below and above the phase transition temperature. Below this temperature, the phospholipids are pushed apart, the packing of the head group is weakened, and the fluidity of the ordered phase is increased. Above the transition temperature, the reduction in freedom of acyl chains causes the membrane to remain condensed and rigidized, with a reduction in area through closer packing and resultant decrease in fluidity. These changes in the fluidity are paralleled by changes in the permeability of the membrane, i.e., depressed by high cholesterol at temperature higher than the T_C° but increased at lower temperatures.

At a ratio of 1:1 of cholesterol to PC, space filling models show consistent and efficient packing of components in two-dimensional lattice in the form of linear arrays, with rows of cholesterol molecules alternating with rows of phospholipids, such that both cholesterol-cholesterol and cholesterol-PC interactions are favourably possible. Thus cholesterol interacts preferentially with the component having the lower transition temperature, i.e., with more fluid phase (and presumably in gauche-confirmation) which is rigidized in the gel phase with a resultant restriction of the transformations of trans-to gauche-conformations. It should be emphasized however, that concentration of cholesterol above 50mole% is difficult to incorporate without disrupting the bilayer configuration and conventional linear structure as it reduce the

number of specific intermolecular interactions. The addition of cholesterol to membranes composed of heteroneous lipids abolishes the phase transitions and alters permeability and fluidity characteristics in the same way.



The micelle forming amphiphiles show relatively high solubility in water. Their self organization in water is the result of hydrophobic effect. The shapes and sizes are characterized by Critical Packing Parameter (CPP).A CPP below 0.5 is reported to give spherical micelles .A CPP between 0.5 and 1 indicates that the surfactant is likely to form vesicles. For cross sectional area of hydrophobic greater than hydrophilic with high curvature such as micelles are formed.

The molecular shape analysis and the concept of shape parameters are very useful for quantitative understanding of the topology of lipid vesicles based on different lipid compositions. However accurate analysis cannot be made because such analysis can only be applied to systems that are at thermodynamic equilibrium .If applied to liposomes containing lipids with a given shape parameter such analysis should yield a very narrow size distribution, rather a quite shallow profile as observed in real practice. Such thermodynamic models also predict the spontaneous formation of liposomes, however a high energy is needed to produce liposomes.

In living cell, billions of vesicles are being generated and reabsorbed constantly. The energy of this process comes from various proteins and their conformational changes. It has been speculated that this energy can be conserved in living cells, where the fusion of vesicle is constantly occurring. The excess energy associated with each vesicles is around 10-15 kT,which corresponds approximately to the energy provided by the hydrolysis of several adenosine tri phosphate molecules. This analysis points to the possibility that some of the energy in the cell can be stored in the curvature of various vesicles and eventually be bioavailable upon vesicle fusion with the membrane.

The typical structural elucidation of PC and FA chains shown in below figure.

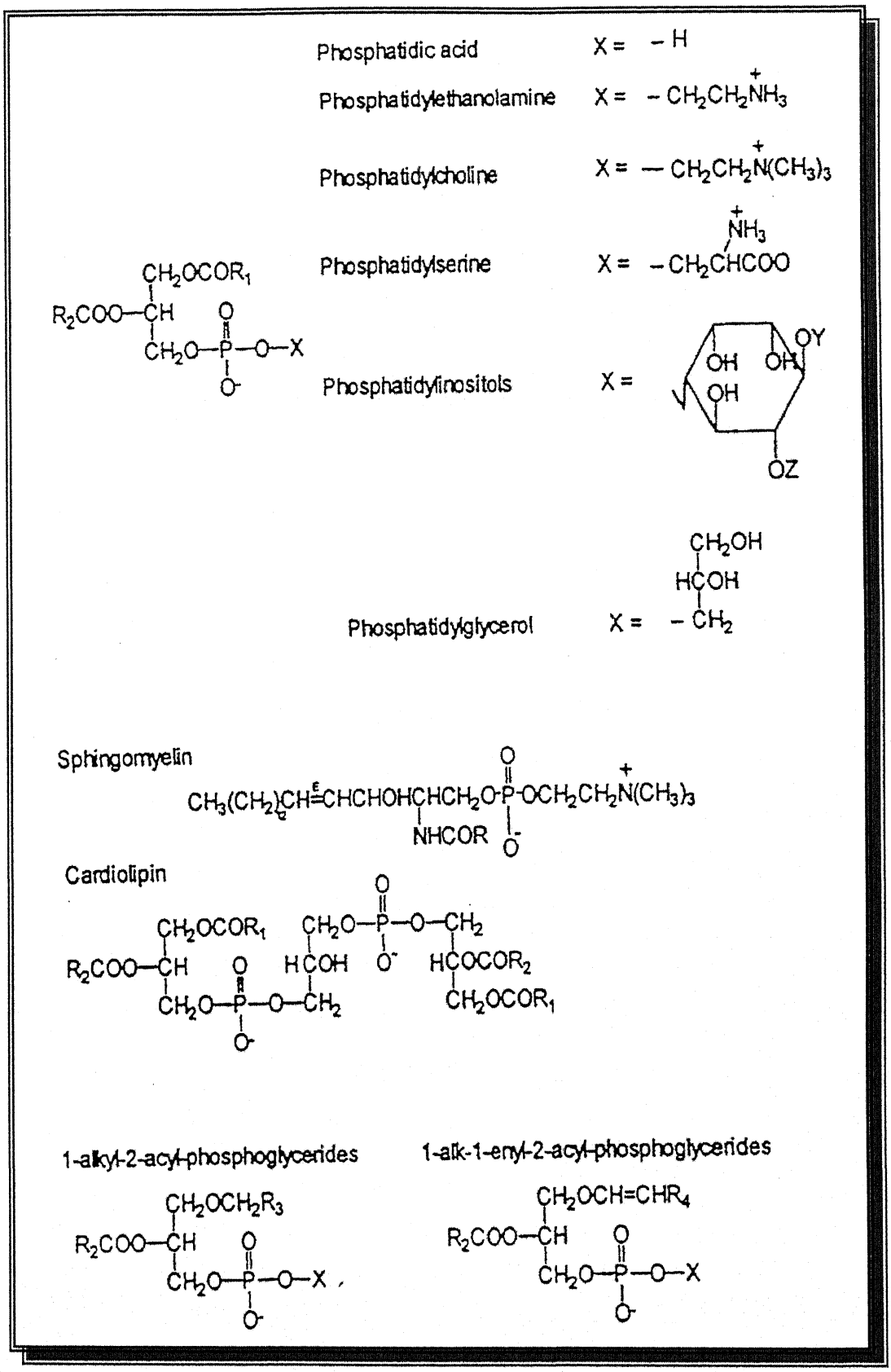


Figure-6: Typical phospholipid and fatty acid chains encountered in liposomes work.

Fatty Acid Chains :

Saturated	Unsaturated
CH_3-$(CH_2)_n$-COO-	CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COO-oleoyl
n= 10 = lauryl	(CC_7C=CC_7COO-oleoyl)
n= 12 = myristoyl	CC_5C=CC_7COO- palmitoleoyl
n= 14 = palmitoyl	CC_4C=CCC=CC_7COO-linoleyl
n= 16 =stearoyl	CCC=CCC=CCC=CC_7COO-linolenyl
n= 20 = arachidyl	CC4C=CCC=CCC=CCC=CC3COO-arachidonyl

Structure of lipids:

Most lipids are composed of tree essential features

1. A polar head group
2. One or more hydrophilic tail chains
3. Back bone structure

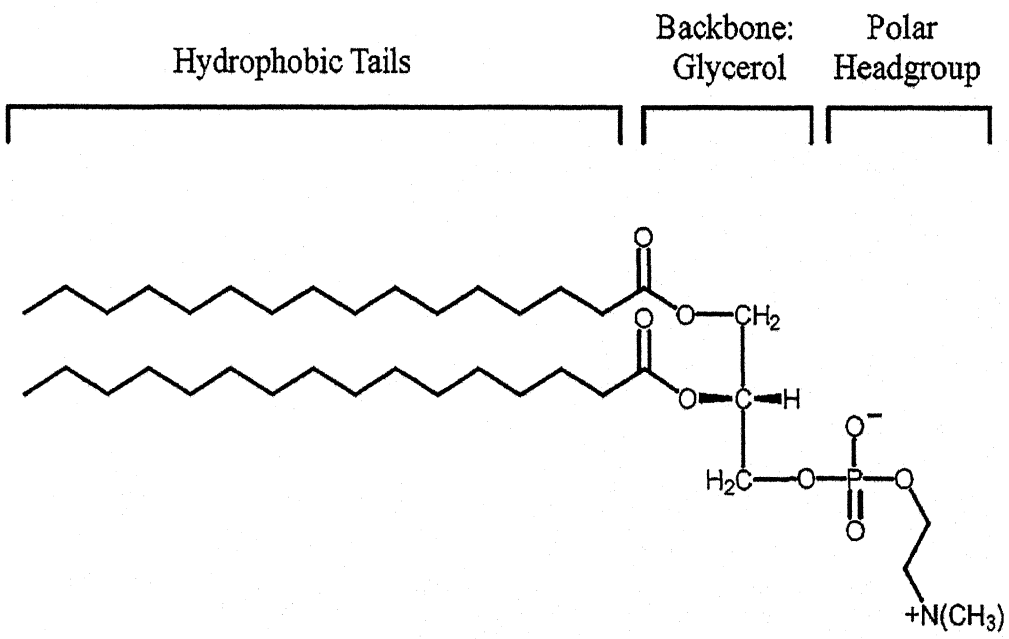


Figure-7: Structural features of lipids, using a glycerophospholipid (phosphatidylcholine) as an example.

STRUCTURE OF THE LIPIDS,

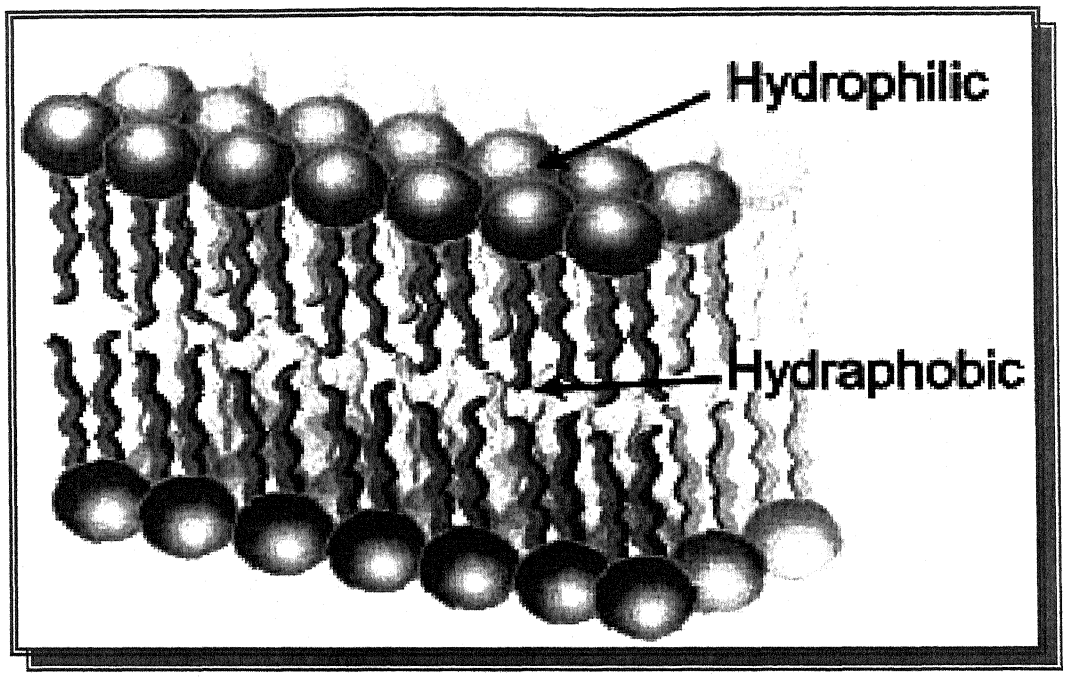


Figure-8: A part showing the hydrophobic and hydrophilic structure in the cell membrane.

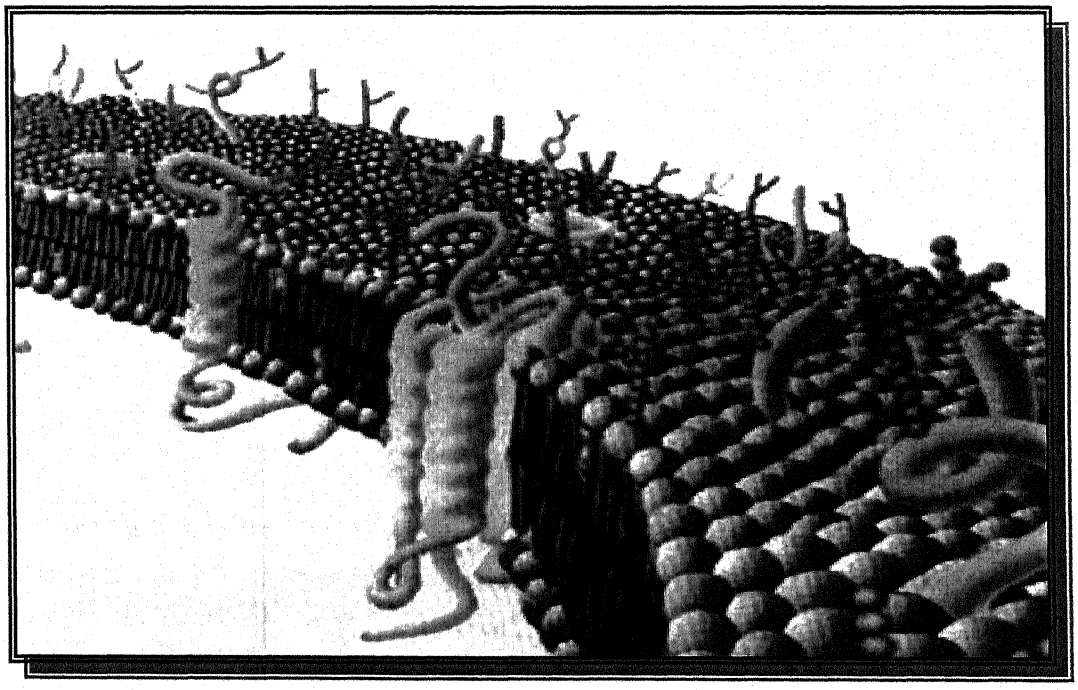


Figure-9: Cell membrane phospholipid.

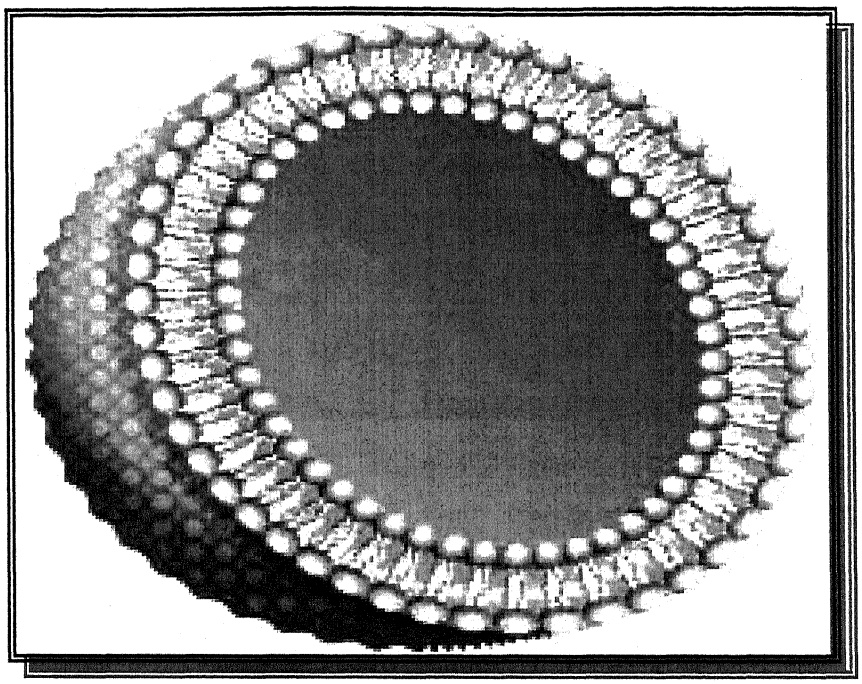


Figure-10: Liposomes are also made up of phospholipids same like cell membrane.

The structure of phospholipid is a marvel. Its two lipid tails (the oil) are hydrophobic (hate water) while the head groups like PC, are hydrophilic (love water). The term amphipathic, which means that the molecule has hydrophobic and hydrophilic sections and preferences. That gives it the ability (or forces it) to self assemble with both oily tails back to back (or tail to tail) hiding from the watery environment. With the head groups looking out on either side of the cell, comfortably sticking their heads in water, the cell has an ingeniously designed protective outer garment. Phospholipids are made up of a head group, a glycerol backbone, and 2 fatty acid lipid tails, 16 to 24 carbons long. The 1940's German method extracted a "Pure Phospholipid" (EPL), which only recently has been duplicated by BodyBio. While the process is more expensive, discarding undesired components from lecithin and raising the phospholipid concentration encourages the formation into a micelle (Liposome).

A micelle/liposome, when exposed to a watery environment forms exactly like cell, a spherical shape, only a thousand times smaller. The phospholipids of micelles and liposomes are identical to cellular phospholipids; however, a liposome is always a bilipid layer and more stable. The tiny sphere can then traverse the gut without being dismembered (it looks exactly like a cell), and not only deliver itself but also

deliver a tiny cargo such as a drug or even another supplement. Today, most pharmaceutical companies are engaged in liposome technology as a drug delivery agent. In the manufacturing process, the drug is trapped inside the sphere so when the liposome touches a cell it is absorbed by endocytosis; the tiny sphere becomes one with the cell membrane and delivers the drug or nutrient inside the cell; an efficient method of taking a drug orally.

The phospholipids are all very similar in structure and composition like cell membrane. The walls of the cell membrane construction is similar like phosphotidyl choline. So that the liposomes reach the cell membrane in the body and they may become accepted as part of the membrane, being the same composition. The ability of phospholipids to acts as the carrier mechanism for delivering active ingredients directly to the cell level by fusion of the cell membrane.

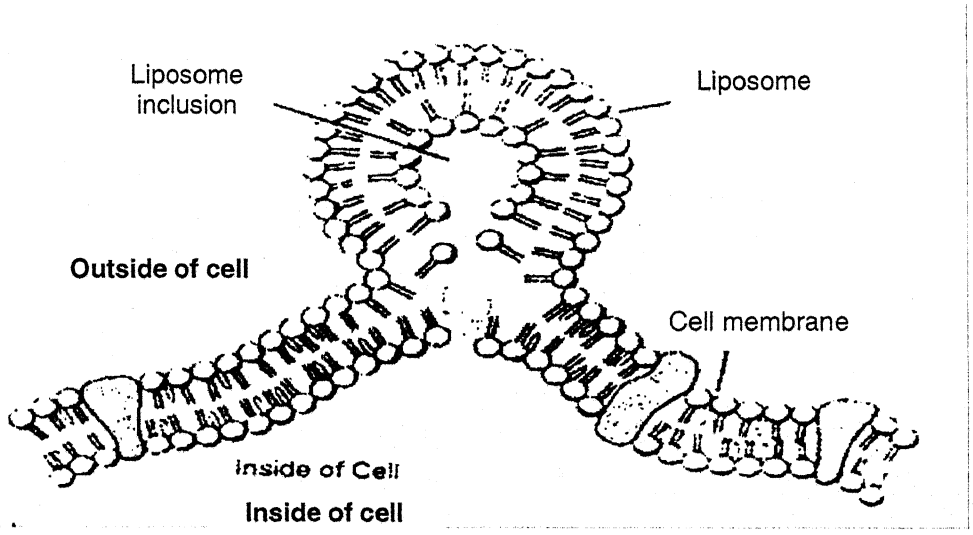


Figure-11: Fusion of liposomes in the Cell membrane.

2.7 SELF ASSEMBLY OF LIPIDS:

Liposomes are microscopic aggregates of highly ordered lipid molecules, which are normally dispersed, in hydrophilic solvent, typically water. The feature of liposomes is that their lipid components are in lamellar arrangements. When any molecules are dispersed in solvent, its fate is dependent on the polarity of the molecule, which reflects the distribution of the electron cloud surrounding it. Polar molecules tend to dissolve in polar solvents such as water; where as nonpolar ones are generally hydrophobic and only dissolve in nonpolar organic solvents. The hydrogen bonds between water molecules allows very small amounts of amphiphile

to dissolve in the free form, but at more appreciable concentration, the hydrophobic residues must be masked from the polar environment[3].When the cross-sectional areas of the polar and nonpolar regions are approximately equal, the molecules is effectively cylindrical in shape and adjacent cylinders are align in a parallel arrangement to form a two dimensional monolayer, with the polar head groups forming one surface of this monolayer and the faty acids acyl chains the other. Two of these monolayers are organized back to back to form a bilayered sheet, in which the hydrophobic regions are sandwiched between the polar group[16].

2.8 MECHANISM OF VESICLE FORMATION:

Liposomes are formed upon hydration of lipid molecules. Normally lipids are hydrated from a dry state (thin or thick lipid film, spray dried powder), and stacks of crystalline bilayers become fluid and swell. Myelin- long, thin cylinders-grow and

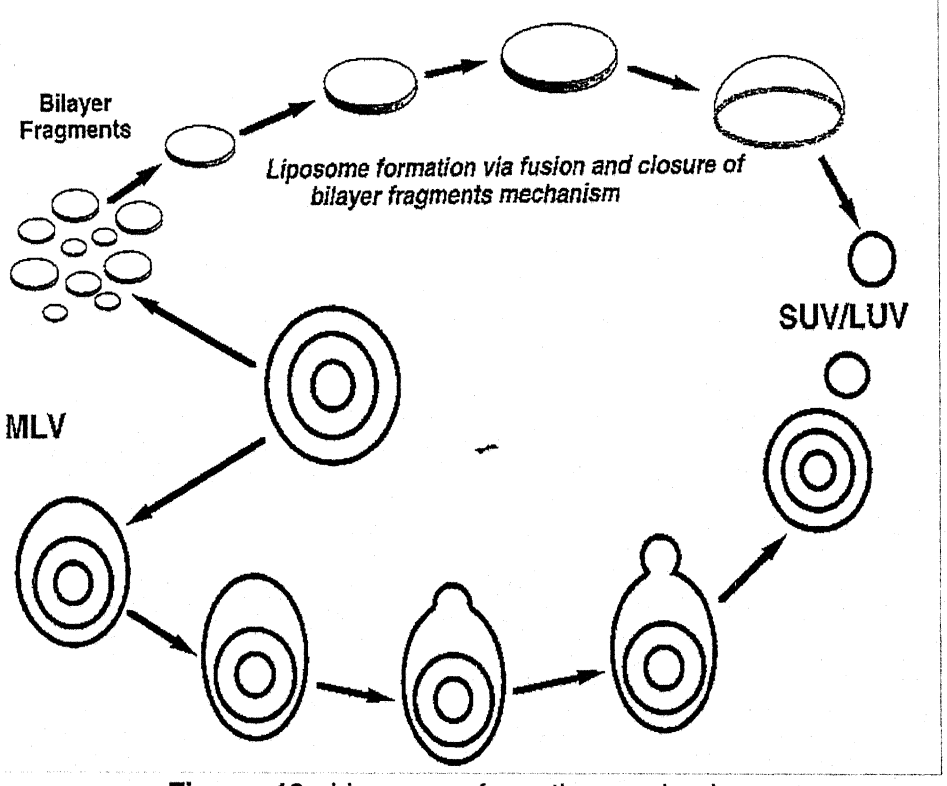


Figure- 12: Liposomes formation mechanism.

upon agitation detach self close in to large, multilammelar liposomes because this eliminates unfavorable interactions at the edges. Once the large particles are formed they can be either broken by mechanical treatments in to smaller bilayered fragments, which close in to smaller liposomes. The size of liposomes in the budding off mechanism is very difficult to calculate, in the self closing bilayer mechanism the

liposomes size depends, the bending elasticity of the bilayer and the edge interactions of open fragments. These factors determine the size of the vesicle size[17].

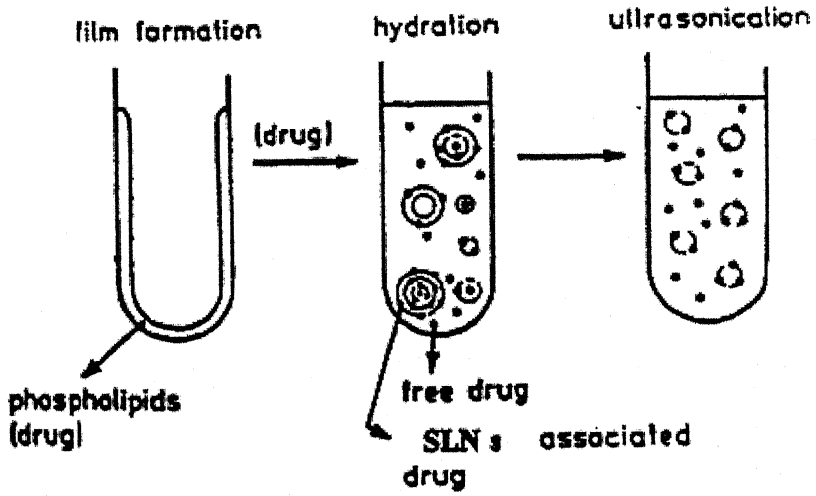


Figure-13: Scheme of the liposomes preparation of liphophillic drugs

2.9 LIPOSOMES CHARACTERISTIC AND QUALITY CONTROL PARAMETERS:

2.9.1 General characteristic studies:

S.No.	Parameters	Technology suggested
1.	pH	pH meter
2.	Conductivity	Conductivity meter
3.	Osmolarity	Osmometer
4.	Phospholipids concentration	Lipid phosphorus content
5.	Phospholipids composition	TLC,HPLC
6.	Cholesterol concentration	Cholesterol oxidase assay, HPLC
7.	Trapped volume	Measure of intraliposomal aqueous phase
8.	Agent concentration Spectrophotometry	Spectrophotometry, HPLC, GC,
9.	Residual organic solvents and heavy metals	NMR, GC
10.	Phospholipids ratio	Determination of drug and Phospholipids concentration
11.	Ion gradient(H^+)	NMR

2.9.2 Physical characterization and stability studies :

S. No.	Parameters	Technology suggested
1.	Vesicle size distribution submicron range	Dynamic light scattering, gel chromatography, electron microscope
2.	Micron size	Coulter counter, light microscopy, light diffraction and specific turbidity.
3.	Electrical surface potential and surface pH	Use of membrane bound electrical field probes and pH sensitive probes.
4.	Zeta Potential	Electrophoretic mobility
5.	Thermo tropic behavior, phase transition, phase separation percentage of free drug	DSC, NMR, FTIR
6.	Percentage of free drug	Gel chromatography, ion exchange chromatography.
7.	Ion gradient	NMR

2.9.3 Chemical stability assay :

S. No.	Parameters	Technology suggested
1.	pH	pH meter
2.	Phospholipids acyl chain auto oxidation	Lipoid peroxides Fatty acid composition, UV/VIS
3.	Phospholipids hydrolysis	TLC, HLC,
4.	Cholesterol auto oxidation	TLC,HPLC
5.	Antioxidant degradation	TLC, HPLC
6.	Agent degradation	TLC, HPLC, Spectrophotometry

2.9.4 Biological assay :

S. No.	Parameters	Technology suggested
1.	Sterility	Aerobic and anaerobic bottle cultures
2.	Pyrogenicity and endotoxin	Rabbit and / or limulus amebocyte lysate test
3..	Toxicity	Related to agent and use of liposomal product
4.	Medium induced leakage	Gel chromatography, ion chromatography.

2.10 LIPOSOMES CLASSIFICATION:

2.10.1 Based on structural parameters :

S. No.	Type	Specifications
1.	MLV	Multilammelar large vesicles > 0.5μm
2.	OLV	Oligolammellar vesicles- 0.1-1 μm
3.	UV	Unilamellar vesicles
4.	SUV	Small unilamellar vesicles-20-100 nm
5.	MUV	Medium sized unilammelar vesicles
6.	LUV	Large unilamellar vesicles- > 1μm
7.	GUV	Giant lamellar vesicles- > 1μm
8.	MV	Multivesicular vesicles

2.10.2 Based on methods of liposomes preparation

1.	REV	Single or oligolamellar vesicles made by reverse-phase evaporation method
2.	MLV-REV	Multilamellar vesicles made by reverse phase evaporation method
3.	SPLV	Stable plurilamelar vesicles
4.	FATMLV	Frozen and thawed MLV
5.	VET	Vesicles prepared by extrusion technique
6.	DRV	Dehydration and dehydration method

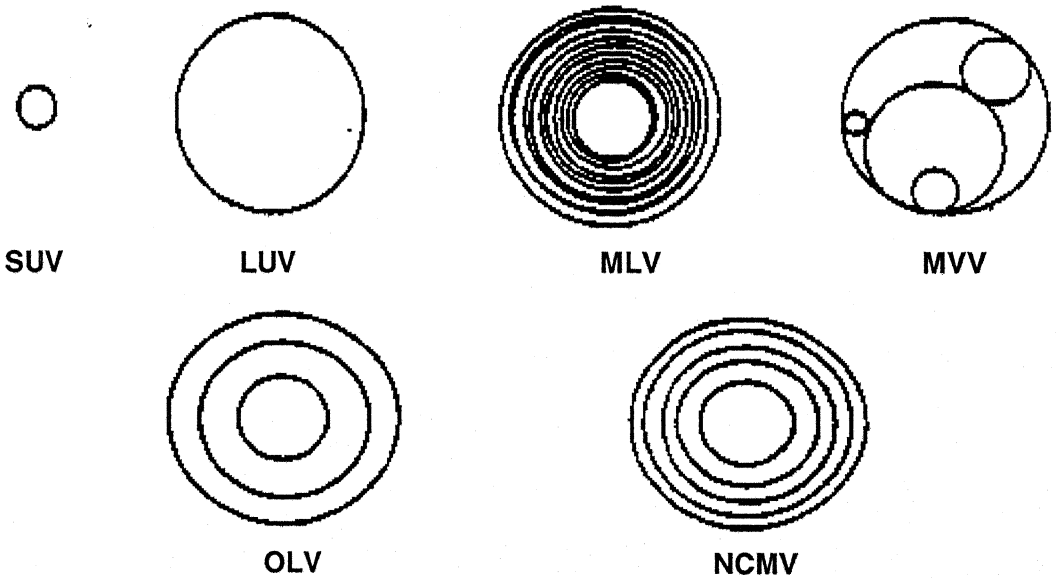


Figure-14: Morphology of different liposomes structures. SUV-Small Unilamellar Vesicles. LUV- Large Unilamellar Vesicles. MLV-Multi Lamellar Vesicles. MVV- Multivesicular Vesicles. OLV-Oligo Lamaller Vesicles. NMLV-Non Classical Multilamellar Vesicles.

2.11 ADVANTAGES OF LIPOSOMES :

- Controlled drug delivery system
- Biodegradable, non toxic
- Carry both water soluble and oil soluble drugs
- Prevention of oxidation
- Protein stabilization
- Controlled hydration.

Liposomes are most useful for being able to transfer and deliver ingredients to the application site. The liposome wall is very similar, physiologically to the material of cell membranes. Liposome delivers actives very specific directly in to the intended cells.

Liposomes as delivery system can be made to release their pay load under a variety of conditions.

- Slow or fast release of hydrophilic drug
- Slow or fast release of hydrophobic drug
- Bilayer composition
- Solvent dependent release
- pH dependent release
- Temperature dependent release

The basic aspects of the drug delivery concepts are :-

- ***Protection***: Active materials are protected by virtue of membrane barrier function
- ***Sustained release:*** Such release is dependent on the ability to vary the permeability characteristics of the membrane by control of bilayer composition and lamelarity.
- ***Controlled release:*** Drug release enabled by exploiting lipid phase transition in response to external triggers such as changes in temperature or pH.
- ***Targeted delivery:*** Liposomes size and surface charge to effect passive delivery to body organs or by incorporating antibodies or other ligands to aid delivery to specific cell types.

2.12 COLLOIDAL STABILITY OF LIPOSOMES:

The entrapment of a drug, the physical stability of a liposome formulation is determined by its colloidal behavior and its ability to retain the cargo for long periods during storage. The liposomes should ideally remain intact upon dilution or changes in the ionic strength, as typically encountered during administration. From a thermodynamically point of view it is notable that plain liposomes, and lipoplexes in particular, are not at equilibrium but represent kinetically trapped systems. Hence,

their structures are relatively stable upon dilution, whereas thermodynamically reversible systems such as micelles and micro emulsions would immediately aggregate or disintegrate[18].

According to the basic DLVO (Derjaguin–Landau–Verwey–Overbeek) theory, a system will be stable in simple electrolyte solutions if the electrostatic repulsion between two particles is larger than their vander Waals attraction. Charged liposomes are thus most suitable stored at low ionic strength, but aggregation may occur at high lipid concentrations or in the presence of multivalent ions with high affinities (e.g.Presence of $Cacl_2$)[19-20]. Coating uncharged colloidal particles with non-ionic hydrophilic polymers, such as PEG, renders them stable against nonspecific interactions and self-aggregation, because the hydration repulsion and the steric barrier prevent close approach. The electrical properties of liposomal surfaces are conveniently examined by micro electrophoresis, which yields the ζ -potential and the surface charge density as characteristic parameters[21]. Even uncharged" liposomes prepared from pure Phosphotidylcholine possesses non-zero -potentials over a wide range of ionic strengths. In most electrolyte solutions the ζ-potential of Phosphotidylcholine tends to be negative due to an anion Layer adsorbed to the raised zwitterionic head group dipoles.

The ζ -potential is sensitive to lipid phase transitions, the adsorption of amphiphile and proteins, steric stabilization with uncharged Poly ethylene glycol and the presence of surface modifications—hence it is a useful parameter for monitoring liposome stability and for checking the reproducibility of batches. Chemical stability of the lipids during storage is another point of concern, especially against hydrolysis, and in the case of unsaturated lipid chains also against oxidation.

Liposomes can be stored frozen or as lyophilized powders, but it is essential to re-check their size distribution, morphology, and entrapped cargo before use. A cryoprotectant, such as trehalose, is suitably added to avoid phase transitions and membrane fusion[22]. To characterize lipid identity, purity, and quantity, MALDI-TOF offers several advantages over conventional chromatographic methods such as TLC, GC, or LC. Normal-phase HPLC is also suitable for analyzing lipid mixtures, which can be detected by evaporative light scattering rather than UV absorption. Similar analytical methods apply to the characterization of lipid-protein and lipid-DNA complexes[23].

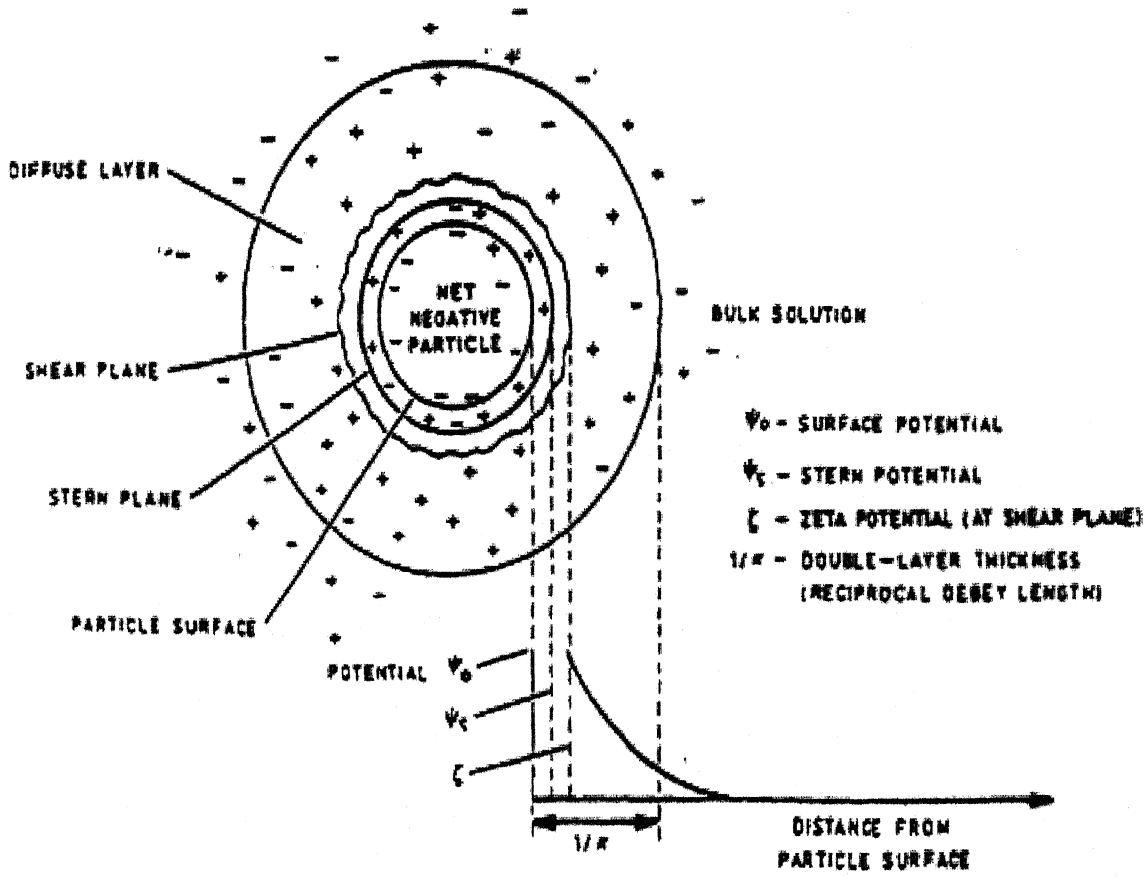


Figure-15: DLVO Potential between colloidal particles.

2.13 APPLICATIONS OF LIPOSOMES:

2.13.1 Liposomes in human therapy:

Despite of the good and encouraging results obtained using liposomes as vehicles for drugs in numerous diseased animal models, in human therapy, the use of liposomes is restricted to systemic fungal infections and cancer therapy, only. However, liposomes based vaccines show great promise and a vaccine against hepatitis A is already on the market.

2.13.2 Liposomes in anticancer therapy:

Based on the early studies that showed that encapsulation of a drug inside of liposomes reduces its toxic side effects, the liposomes were considered as attractive candidates for the delivery of anticancer agents. However, their use was hampered by the rapid uptake of conventional liposomes by MPS cells. The increase of in vivo circulation time of modified lipids (PEG polymerized lipids, gangliosides, shingomyelin etc.) restored the initial expectation of the advantages of liposomes.

Intravenously administered stealth liposomes were passive targeted to solid tumors due to their extravasation in leaky blood vessels supporting the tumor[24]. The good results obtained with liposomal-encapsulated doxorubicin and daunorubicin have lead to two products licensed for use in the treatment of Kaposi' sarcoma, namely Doxil and Daunoxome. Doxil (commercialized by Sequus Pharmaceuticals, Menlo Park, USA) is a suspension of doxorubicin precipitated in 80-100 nm sterically stabilized liposomes. Daunoxome (commercialized by NeXstar Pharmaceuticals, Inc., Boulder, USA) is a small, rigid formulation of liposomes with daunorubicin. These liposomes circulate in the vasculature of patients for several days, and thus have increased chances of extravasating at sites of increased permeability. The success achieved with anthracycline anticancer agents led to the development of other liposomal formulations that are in preclinical stages (5-fluorouracil lipid analogue[25] vincristine [26] a porphyrin derivative for use in combination with laser light irradiation , bleomycin[27], paclitaxel[28], valinomycin in combination with cisplatin[29].

2.13.3 Liposomes in infection treatment:

Due to their uptake by the cells of the MPS, mainly Kupffer cells and spleen macrophages, conventional liposomes are useful in the treatment of parasitic infections of the MPS, such as leishmaniasis. Encapsulating the amphotericin B into liposomes reduces the renal and general toxicity, and the therapeutic efficiency is improved. Ambisome is a formulation of small, negatively charged liposomes with amphotericin B licensed for clinical use and commercialized by NeXstar, Pharmaceuticals Inc., Boulder, USA[5]. Now, the attention is focused on the encapsulation of more powerful antibiotics (that are exceedingly toxic in free form) and on the develop development of liposomal formulations for delivering the drugs to other sites than MPS. The encapsulation of the anti-tuberculosis drug rifampicin or isoniazid in liposomes targeted to lung improves the efficacy of the drug.[30]

2.13.4 Liposomes as vaccine system:

Liposomes can be used as enhancers of the immunological response by incorporation of antigens 61. For this purpose the liposomes are administered intramuscularly, a location where the encapsulated antigen is released slowly and accumulate passively within regional lymph nodes. To control the antigen release and to improve the antibody response, the liposomes encapsulating antigens are

subsequently encapsulated into alginate lysine microcapsules[31]. At present, Epaxal, a liposome-based vaccine against hepatitis A was licensed for clinical use and was introduced on the market by Swiss Serum and Vaccine Institute, Bern, Switzerland[32]. This vaccine contains formalin inactivated hepatitis A virus particles attached to phospholipid vesicles together with influenza virus haemagglutinin. Hepatitis A virus incorporated into liposomes proved to be a suitable formulation in term of rapid seroconversion, high level of mean antibody content and low reactogenicity[33]. Also, there are in clinical trial vaccines against influenza, hepatitis B, diphtheria, tetanus, e-coliinfection.

2.13.5 Liposomes in gene delivery:

Gene therapy is the process which DNA delivers sequences encoding specific altered genes to cells with the goal of treating or curing genetic diseases. Thus, instead of treating the symptoms of the disease, as in conventional medicines, gene therapy has the potential to correct the underlying cause of genetic diseases. While the idea of gene therapy is a simple concept, the delivery of genes to the diseased areas turned out to be a difficult task. The problems associated with the use of viral vectors for gene therapy, lead to the search for less-hazardous, nonviral delivery systems. As an alternative to viral vectors, cationic liposomes have been developed for gene transfer since they have no limit for the size of the genes to be delivered and exhibit low immunogenicity. The efficacy of this system has been limited by the non-specific adherence to many cell types. In order to obtain an effective DNA transfer it is necessary to administer liposomes to a site near to the target area. The use of ligand-targeted liposomes will make possible to direct them precisely to diseased cells and not to other cells. Some pharmaceutical companies (Vical Company, San Diego, USA, Targeted Genetics Corporation, Seattle, USA, ValentisR Burlingame, USA) are engaged in liposome- based gene delivery and have products in clinical trials. Vical Company (San Diego, USA) has two compounds on trial that are based on liposomes for gene delivery: (i) Allovectin-7: liposomes carrying a gene for HLA-B7 (a highly immunogenic molecule) that are injected into tumors: this is in phase III trial for metastatic melanoma and phase II trial for patients with head and neck squamous cell carcinoma and (ii) Leuvectin, a DNA/lipid complex containing gene for IL-2, a immunostimulatory cytokine, that is in phase II trial for patients with prostate cancer. ValentisR Company (Burlingame, USA) has a

liposome-based system in phase I trial for gene therapy with Del-1 (Developmentally Regulated Endothelial Locus-1, an extracellular matrix protein involved in the early growth and development of blood vessels and bone) for the treatment of peripheral arterial disease and ischemic heart disease.

A cationic liposome /E1A complex (a gene from common cold virus that acts as tumor inhibitor) that is injected intratumoral, is under investigation by Targeted Genetics Corporation (Seattle, USA); the liposome complex is in phase II study in the treatment of patients with recurrent head and neck squamous cell carcinoma [34]and in phase I in ovarian cancer in combination with paclitaxel (Taxol) and cisplatin chemotherapy[35]. Intratumoral injections of liposome/ E1A complex were safe and well tolerated. The E1A gene expression was accompanied by HER- 2/neu down regulation, increased apoptosis, and reduced proliferation. Also in clinical trials, the UK CF Gene Therapy Consortium (London, United Kingdom) employs the complexes liposome/gene CFTR (cystic fibrosis transmembrane conductance regulator) that is delivered as an aerosol to the nose and lung of patients with cystic fibrosis[36].

2.14 AN INTRODUCTION TO HYDROGELS AND ITS APPLICATIONS:

Hydrogels are cross-linked macromolecular gel networks that swell in water or biological fluids[37]. The cross links can be formed physically or chemically. Covalent bonding between the polymer chains bonds chemical cross Links. Weaker forces such as Vander Waals forces and hydrogen bonds form physical cross-links. Semi crystalline. Uncross-linked hydrophilic polymers may form hydrogels. Since, the crystallites act as physical cross-links and do not dissolve in water[38]. A few examples of polymer that can form hydrogels include HPMC, Carbopol, polaxamer, Chitosan.

2.14.1 Properties of hydrogels:

A. Equilibrium swelling:

The most significant property of hydrogels is the degree of equilibrium swelling, expressed as the amount of water absorbed by the gel, relative to the dry volume (or mass) of the hydroge1[39]. The amount of water absorbed, varying from 20%to often over 90% water by weight[40], is a function of the hydrophilicity of the polymer. The network structure and the number of ionized groups on the polymer. The theoretical bias for gel swelling has been developed which assumes that the swelling of gels is the result of several independent free-energy changes that occur when the gel is mixed with the solvent, if the gel contains ionizable groups such as carboxyl groups. Polyelectrolyte gels normally swell much more than nonionic gels due to the presence of ionic charges. This means that the donnan effect[41], while electrostatic repulsion of like charges on the polymer network can generally be ignored. There are two sources of ions inside the gel one source is the dissociation of ionic groups bound to the polymer network. The other source is the diffusion of ions into the gel from the surrounding solution the concentration of ions inside the gel is typically greater than that of the external solution As a result, water flows into the gel to dilute the ions, resulting in gel swelling.

B. Solute permeation:

Hydrogels have been classified in three basic categories based on the size of the pores[42]. Macro porous hydrogels have large pores ranging from 50 nm to 1000nm. Convection is believed to be the predominant mass transfer mechanism in

macroporous hydrogels. Microporous hydrogels have pore sizes ranging from *5* nm to *50* nm. Diffusion of solutes in microporous hydrogels occurs in water-filled pores. The structure of which influences the mass transfer process. Nonporous hydrogels, as their name implies, do not have a porous structure. Molecular diffusion in nonporous hydrogels occurs in the spaces between the macromolecular chains. Convection is usually negligible in nonporous hydrogels. Solute permeation through hydrogels may be determined by various mechanisms such as diffusion, convection, and osmosis. Fick's first law can describe the transport of solutes through the hydrogels by diffusion[43]. Solute moves according to the concentration gradient i.e. down hill transport (Higher concentration to the lower concentration).

There are two mechanisms proposed to explain diffusion through the hydrogels, the pore and partition mechanisms. The pore mechanism assumes that the solute moves through the bulk water phase of the swollen polymer. A hypothetical network of pores within the hydrogels has been suggested since the diffusion is affected by the size of the solute involved[44]. The partition mechanism is assumed to involve the dissolution of the solute into the polymer structure. Therefore, this mechanism is affected by the interactions between the polymer and the solute. Various models have been proposed to describe solute diffusion within polymer networks in relation to polymer hydration. The most successful and most widely used model is the free volume theory proposed, which assumes that the solute moves only within the water filled regions of the gel. The partition behavior of charged solutes in polyelectrolyte gels. When solutes carry the opposite charge of the network, ion exchange occurs between the solutes and the counter-ions of the network, resulting in substantial sorption. The partition coefficient increases depending on the ionic equilibrium constants. When solutes carry the same charge as the polymer network, Donnan ion exclusion causes the solutes to be excluded from the gel so that the partition coefficient of the solute is less than one. Hydrophobic interactions are important for non-polar solutes in gels with hydrophobic regions. Such interactions may be predicted by the octanol-water partition coefficient, which is available for a variety of drugs.

C. Biocompatibility:

Biocompatibility is a requirement for any device or material to be placed in Intimate contact with the Living tissue or biological fluids. If the material is not biocompatible, there could be adverse immune responses, tissue damage, toxic or allergic responses, Carcinogenesis, Thrombosis, depletion of electrolytes and ion alteration of plasma proteins and enzymes. Hydrogels are usually biocompatible[45]. The Chitosan, HPMC, Polaxamer, Carbopol and their copolymers are found to be biocompatible and degradable in their physiological environment yielding naturally occurring metabolic products. A transductional protein-based polymer composed of repeating peptide sequences has also been found to exhibit good biocompatible inmutagenicity, antigencity, and toxicity and cell attachment evaluations. Some studies have tried to relate the biocompatibility of hydrogels with their inherent properties, such as water content, surface charge and surface tension. However, some of the results are ambiguous and do not support a direct correlation of the physical properties with the biocompatibility.

D. Applications of hydrogels:

Hydrogels have been investigated for many pharmaceutical applications. For example, they have ken fabricated into devices for controlled drug delivery. As a result of their swelling properties, hydrogels present useful carriers for controlled drug release. Also, their relative hydrophilic character provides a stable environment for the loading of macromolecules such as proteins and peptides. Hydrogels devices have been classified based on their mechanisms of drug release, namely, diffussion-controlled, chemically controlled and solvent-activated devices[46]. Stimuli-sensitive hydrogels have been developed for regulated drug release by external stimuli, e.g. temperature[47]. Bioadhesive hydrogels have been studied for sustained retention of the device within the body as well as controlled release of drugs. Hydrogels have also been used as biomedical materials. They have been investigated for use as blood contact materials. Heparinization of hydrogels bas been carried out to prevent unwanted coagulation activity in the body. Hydrogels have been used as contact lens materials. As some "smart" hydrogels can transform electrochemical stimuli into mechanical work, their function as artificial muscle tissues has been studied. These artificial muscles may have applications in medical implants, prosthetic muscles or

organs and robotic grippers. The mechanical properties of the hydrogels have been used towards the development of an artificial tendon. Bioerodible hydrogels have also been applied as sutures, or to guide proper healing of body tissue after surgery a process known as guided tissue regeneration[48].

2.15 MODELING OF RELEASE PROFILE:

In recent years, drug release from pharmaceutical dosage forms has been the subject of intense and profitable scientific developments. Whenever a new dosage form is developed form is developed, it is it is necessary to ensure that drug release occurs in an appropriate manner. The quantitative analysis of the values obtained in release tests is easier when mathematical formulas that express the release results as a function of some of the dosage forms characteristics are used. In some cases, these mathematic models are derived from the theoretical analysis of the occurring process. Drug dissolution from dosage forms has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or $Q=f(t)$. Some analytical definitions of the $Q(t)$ function are commonly used, such as zero order, first order, Hixson–Crowell, Weibull, Higuchi, , Korsmeyer–Peppas.

In vitro dissolution has been recognized as an important pharmaceutical dosage form can influence the release element in drug development. Under certain conditions it kinetic be used as a surrogate for the assessment of Bioequivalence. Several theories / kinetics models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where f_t is a function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. In most cases, with tablets, capsules, coated forms or prolonged release forms that theoretical fundament does not exist and some times a more adequate empirical equations used. A water-soluble drug incorporated in a matrix is mainly released by diffusion, while for a low water-soluble drug the self-erosion of the matrix will be the principal release mechanism. To accomplish these studies the cumulative profiles of the dissolved drug are t more commonly used in opposition to their differential profiles. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used.

2.15.1 Zero order kinetics:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the following equation

W_o-W_t= Kt

W_o is the initial amount of drug in the dosage form.

W_t is the initial amount of drug in the dosage form at time(t)

K is the proportionality constant.

Dividing this equation by and simplify

Ft=Kot

Where ft= 1-(W_t-W_o) and ft represents the fraction of drug dissolved in time t and K_o the zero order of release constant.

This relation can be used to describe the modified release dosage form; the following relation can, in simple way to express this model

$Q_t = Q_o + K_o t$

Q_t is the amount of drug dissolved in time t.

Q_o is the amount of drug in the solution.

2.15.2 First order kinetics:

Gibaldi and Feldman first proposed the application of this model to drug dissolution studies in 1967 and later by Wagner in 1969. The dissolution phenomena of solid particles in a liquid media implies a surface action, as can be seen by the Noyes- Whitney equation

DC/dt= K (Cs-C)

C is the concentration of the solute in time t

Cs is the solubility in the equilibrium at expression temperature

K is the first order proportionality

Hixson and Crowell adapted the Noyes-Whitney equation in the following manner.

DW/dt= Ks (Cs-C)

W is the amount of solute in solution at time t,

DW/dt is the passage rate of the solute in to solution in time t

K is a constant

Hixson crowell equation can be rewritten as

DW/dt = Ks/v(VCs-W) = K(VCs-W)

K=K1s

If one dosage form with constant area is studied in ideal condition (sink conditions), it is possible to use this last equation that after integration, will become

W= VCs (1-e^{-kt})

This equation can be transformed, appliii9ng decimal logarithm in both terms in to

log (VCs-W)= log VCs-kt/2.303

In decimal logarithms

Log Qt= log Qo+k1t/2.303

Qt is the amount of drug released in time t

Qo is the initial amount of drug in the solution

K1 is the first order constant.

2.15.3 Higuchi model:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drops incorporated in the matrixes. The drug particles dispersed in a uniform matrix behaving as the diffusion media, the relation obtained was the following:

f_t = Q=√D(2C-C_s) C_st

Q- is the amount of drug released in time t, per unit area,

C – is the drug initial concentration,

C_s – is the drug solubility in the matrix media.

D – is the diffusivity of the drug molecules (diffusion constant in matrix).

$dQ = Cdh - ½ (C_s dh)$

but, in accordance to the first law (dq/dt = DC/h)

Higuchi in 1962 proposed the following equation, for the case in which the drug is dissolution.

In general way it is possible to resume the Highuchi model to the following expression (generally known as the simplified Higuchi model).

$F_t = K_H t^{1/2}$.

Where K_H is the Highuchi dissolution constant treated sometimes in a different manner by different authors and theories. Higuchi describes drug release as a diffusion process based on Fick's law, square root time dependent.

2.15.4 Hixson Crowell model:

Hixson Crowell in 1931 recognizing that the particle regular area is proportional to the cubic root of its volume derived an equation that can be described in the following manner.

$W_o^{1/3} - W_t^{1/3} = K_s^t$.

W_o is the initial amount of drug in the dosage form.

Wt, is the remaining amount of drug in the dosage form at time t.

K_s is a constant incorporating the surface-volume relation.

Dividing by $W_o^{1/3}$ and simplify.

$(1-f_t)^{1/3} = 1-K_B^t$

$f_t=1-(W_t/W_0)$

f_t - represent the drug dissolved fraction at time t and K_B is release constant.

This model has been used to describe the release profile keeping in mind the diminishing surface of the drug particles during the dissolution.

2.15.5 Korsmeyer – Peppas model:

Korsmeyer *et al.*, in 1983 was developed a simple, semi empirical model, relating exponentially the drug release to the elapsed time (t).

$f_t = at^n$

a – is a constant incorporating structural and geometric characteristics of the drug dosage from, n is the release exponent, indicate of the drug release mechanisms and the functions of t is M_t/M_∞ (Fractional release of drug).

$$= D \frac{\partial c}{\partial t} \frac{\partial^2 c}{\partial x^2}$$

D -is the drug diffusion coefficient (Concentration independent). If drug release occurs under perfect sink condition, the following initial and boundary conditions, the following initial and boundary conditions can be assumed.

$t=0-d/2<x<d/z$ $C = C_0$

$t>0.$ $x = \pm d/z$ $C = C_1$

C_0-is the initial drug concentration in the device.

C_1 -is the concentration of drug at the polymer water interface.

In the diffusion is the main drug release mechanism, a graphic representation the drug amount released, in the referred conditions, versus the square root of time should originate a straight line. Under some experiment situations from fick equation. In these cases a more generic equation can be used[48]

$$\frac{M_t}{M_\infty} = at^n$$

Mathematical models used to describe drug release curves.

1.	Zero order	$Q_t = Q_o + K_o t$
2.	First order	$\ln Q = \ln Q_o + K_1 t$
3.	Second order	$Q_t / Q_\infty = (Q_\infty - Q_t) K_2 t$
4.	Hixson- Crowell	$Q_o^{1/3} - Q_t^{1/3} = K_s t$
5.	Weibull	$\log[-\ln(1-(Q_t/Q_s))] = b \times \log t - \log a$
6.	Higuchi	$Q_t = K_H \sqrt{t}$
7.	Korsmeyer- Peppas	$Q_t / Q_\infty = K_k t^n$

Chapter-3

Literature Review

LITERATURE REVIEW

Marie Mougin- Degraef et al[49]., A new method to label preformed liposomes with high activities of radio halogenated compounds has been developed. It uses activated esters of simple synthetic molecules that may be readily halogenated, such as Bolton-Hunter reagent (BH), and arginine-containing liposomes. Under optimized conditions, between 37 and 55°C, 62 ± 4% (mean ± SD) of radiolabeled BH were consistently encapsulated in the liposomes within 30 min. In molar amounts, this corresponds to a mean of 56 n mol of BH per ìmol of lipids. Based on achievable specific activity, up to 2.8 GBq of iodine-131 could be entrapped per ìmol of lipids. Leakage of radioactivity was very low, with less than 5% of the encapsulated activity released within 6 days at 4°C in phosphate- buffered saline and less than 50% within 24h in human serum at 37°C. The labeling stability, and the fact that both conventional and PEGylated liposomes can be readily labeled with high doses of radioactivity, will make this technique useful for in vivo targeting applications, such as tumor detection (using iodine-123 or iodine-124) or therapy (with iodine-131 or astatine-211).

Loan Honeywell-Nguyen P et al[50]., The main problem in delivery of drugs across the skin is the barrier function of the skin, which is located in the outermost layer of the skin, the stratum corneum. The stratum corneum consists of corneocytes surrounded by lipid layers, the so-called lipid lamellae. When applying drugs onto the skin, the major penetration pathway is the tortuous intercellularroute along the lipid lamellae. In order to increase the number of drugs administered via the transdermal route, novel drug delivery systems have to be designed. Among these systems are iontophoresis, electroporation, microneedles, and vesicular systems.

Andreas Wagner et al[51]., new scalable liposome production system is presented, which is based on the ethanol injection technique. The system permits liposome manufacture regardless of production scale, as only free disposable vessel volumes determine scale. Once the parameters are defined, just just changing the process vessels can perform an easy scale up. These vessels are fully sterilizeable and all raw materials are transferred into the sanitized and sterilized system via 0.2 µm filters to guarantee an aseptic production. Liposome size can be controlled by the

local lipid concentration at the injection point depending on process parameters like injection pressure, lipid concentration and injection rate. These defined process parameters are furthermore responsible for highly reproducible results with respect to vesicle diameters and encapsulation rates Compared to other technologies like the film method which is normally followed by size reduction through high pressure homogenization, ultrasonication or extrusion, no mechanical forces are needed to generate homogeneous and narrow distributed liposomes.

Shantha et al[52]., Liposomal delivery systems for water-soluble bioactives were prepared using the proliposomeand the microfluidization technologies. Iron, an essential micronutrient asferrous sulfate and ascorbic acid, as an antioxidant for iron were encapsulated in the liposomes. Liposomes prepared by the microfluidization technology using 6% (w/w) concentration of the lipid encapsulated with ferrous sulfate and ascorbic acid had particlesize distributions around 150 to 200 nm, whereas liposomes from the pro-liposome technology resulted in particle sizes of about 5 m. The encapsulation efficiency of ferrous sulfate was 58% for the liposomes prepared by the microfluidization using6% (w/w) lipid and 7.5% of ferrous sulfate concentrations, and it was 11% for the liposomes from pro-liposome technology using 1.5% (w/v) lipid and 15% of ferrous-sulfateconcentration. Both the liposomes exhibited similar levels of oxidative stability, demonstratingthe feasibility of microfluidization-based liposomal delivery systems for large-scale food/nutraceutical applications.

Volkmar Weissig et al[53]., Mitochondrial research is presently one of the fastest growing disciplines in biomedicine. Since the early 1990s, it has become increasingly evident that mitochondrial dysfunction contributes to a large variety of human disorders, ranging from neurodegenerative and neuromuscular diseases, obesity, and diabetes to ischemia-reperfusion injury and cancer. Most remarkably, mitochondria, the "power house" of thecell, have also become accepted as the "motor of cell death" reflecting their recognizedkey role during apoptosis. Based on these recent exciting developments in mitochondrial research, increasing pharmacological efforts have been made leading to the emergence of "Mitochondrial Medicine" as a whole new field of biomedical research. The identification of molecular mitochondrial drug targets in combination with the development of methods for selectively delivering biologically active molecules to the site of

mitochondria will eventually launch a multitude of new therapies for the treatment of mitochondria-related diseases, which are based either on the selective protection,repair, or eradication of cells. Yet, while tremendous efforts are being undertaken to identify new mitochondrial drugs and drug targets, the development of mitochondria-specific drug carrier systems is lagging behind. To ensure a high efficiency of current and future mitochondrial therapeutics, colloidal vectors, i.e., delivery systems, need to be developed able to selectively transport biologically active molecules to and into mitochondria within living human cells. Here we review ongoing efforts in our laboratory directed toward the development of different phospholipid- and nonphospholipid-based mitochondriotropic drug carrier systems.

Montazer, M et al[54].,Liposomes are lipid vesicles that are composed of amphiphile molecules and can carryhydrophobic and hydrophilic materials. In this research work liposomes used as carrierfor transfer of dye molecules into wool fibers. The preparation and production ofmultilamellar liposomes (MLV) from Soya lecithin were carried out and the behavior of liposomes at different temperature was studied. The effect of different concentration of liposomes in the dye exhaustion profile of two dyes (Namely, Irgalan Blue FBL and Lanaset Blue 2R) at two different temperatures of 85°C and 95°C on the wool fabric was investigated. The results showed that presence of liposomes in the dye-bath helps to increase the dye absorption on the wool fabric before 80°C. Dyeing at higher temperature and longer time leads to a decrease in the final exhaustion along with increase in the liposomes concentration. Liposomes at high temperature converted to the disperse phospholipids unimers that may deposited on the fabric surface and may produce a hydrophobic barrier against absorption of dye. The presence of 1% o.w.f.(on weight of fabric) of liposomes at 85°C improved the dye exhaustion of Irgalan BlueFBL on the wool fabric. The wash fastness properties of samples which dyed in thedye-bath containing liposomes also improved.

Elena Alexopoulou et al[55]., Liposomes composed of egg-phosphatidylcholine (EPC) incorporating quercetin (QR)were prepared by the thin-film hydration method (TFHM) and the monophase solutionmethod (MSM). A rapid and slow freeze-drying process was applied for both laboratoryand industrial scales. The purpose of this study was to compare the twomethods of liposome preparation, and further determine whether the lyophilizationprocess affects the liposome

physicochemical characteristics (size, polydispersityindex, and z-potential) and incorporation of quercetin.

Waraporn Suwakul et al[56]., Propylthiouracil, a lyophobic drug with an antiproliferative activity, was formulated into niosomes using various classes of nonionic surfactants. Feasibility of vesicle formationby the sonication method was evaluated. Size and size distribution was measuredby laser diffraction. Entrapment and drug release over 24 h were monitored byUV spectrophotometric method at 275 nm. The results revealed that niosomes readilyformed from various compositions of nonionic surfactant and cholesterol, with or withouta stabilizer. Entrapment of PTU in niosomes depended on bilayer composition. Therelease of PTU from all niosomal formulations studied was retarded and followed thefirst-order kinetics. Degree of slow release had a negative correlation with drugentrapment. The release rate also depended on the physical state of the bilayer. Theresults of this study indicate that PTU niosomes were able to control the release of PTU and might be of value to develop further into topical formulations.

Murakami,T et al[57]., Trapped bovine serum albumin (BSA), were modified withdiglucosamine by two methods. The liposome was prepared by a freeze-thawingmethod in the presence of the disaccharide, or the disaccharide was added to the liposomeprepared in advance without it. To examine the effects of diglucosamine, themorphology, mean particle size, and zeta potential of both liposomes were compared with those of BSA-entrapping liposome prepared without the disaccharide. Diglucosaminecaused no remarkable change in shape and no aggregation of the liposome.The presence of the disaccharide was confirmed on the surfaces of modified liposomes,and the entrapment of BSA into the liposomes was increased by the disaccharide. Theentrapment behavior was affected by the way the disaccharide was added, and the difference in the way the BSA was entrapped was also indicated.

Katare O P *et al[58].,* studied that the tamoxifen, an anti-estrogen compound,has recently been figured as a useful agent in the treatment of certain skin specific disorders. This recent found application has generated an interest in its topical formulation in order to avoid the side effects associated with oral administration, while parenteral administration is restricted due to its limited aqueous

solubility. Liposomal carriers, well known for their potential in topical drug delivery, have been chosen to help transport tamoxifen molecules in the skin layers.

Lars Ingebrigtsen., Martin Brandl[59]., Studied that the, small Liposomes obtained by high-pressure homogenization were fractionated according to their particle sizes by size exclusion chromatography (SEC). The subfractions were analyzed by photon correlation spectroscopy (PCS) as well as enzymatic phosphatidylcholine (PC) assay for their particle sizes and lipid contents, respectively.

Hyde, S. C *et al*[60]., studied that the major cause of mortality in patients with cystic fibrosis(CF) is lung disease. Ten subjects received plasmid DNA expressing the CFTR cDNAcomplexed with DC-Chol/DOPE cationic liposomes. Each subject received three doses, administered 4 weeks apart. There was no evidence of inflammation, toxicity or an immune response.

Marilena Celano *et al*[61]., concluded that the findings show that, in vitro against human thyroid cancer cells, the gemcitabine incorporation within liposomes enhances the drug cytotoxic effect with respect to free gemcitabine, thus suggesting a more effective drug uptake inside the cells.

Brandl M *et al*[62].,Studied with the highly concentrated, semisolid phospholipid dispersions. Their preparation is based an `forced hydration' of (phospho-)lipid(s) by high-pressure homogenisation in the presence of relatively low amounts of water. The inner structure of the obtained emisolid pastes, as revealed by freeze-fracture electron microscopy, is bestdescribed as a matrix of densely packed vesicles. Depending an the lipid content the characteristics of these vesicles rangefrom very homogeneous, Small and unilamellar to more heterogeneous in size as well as lamellarity.

Lorraine D. Hernandez *et al*[63]., studied that the membrane fusion proteinsthat function at neutral pH, for example the humanimmunodeficiency virus envelope (Env) glycoproteinand intracellular fusion machines, are activated fortarget bilayer binding. We have addressed this questionusing a soluble oligomeric form of an avian retroviralEnv glycoprotein (API) and soluble forms of its receptor.

Zhao min zheng et *al*[64]., studied that the pharmacokinetics and biodistribution of cisplatin encapsulated in polyphase liposome (KM-1) werecompared with those of free drug in rats.

Subeet Jain *et al*[65]., studied that the present investigation aimed at formulation, performance evaluation, and stability studies of new vesicular drug carrier system protransfersomes for transdermal delivery of the contraceptive agent, levonorgestrel. Protransfersome gel (PTG) formulations of levonorgestrel were prepared and characterized for vesicle shape, size, entrapment efficiency, turbidity, and drug permeation across rat skin and were evaluated for their stability.

Carla Andreria Miranda da Costa *et al*[66]., studied that the different methodologies for the preparation of Dipalmitoylphosphatidylcholine /cholesterol (DPPC/Chol) liposomes entrapping theanticancer agent 5-fluorouracil (5-FU), a drug usually employed in melanoma therapy, designed for topical administration.

Hagen von Briesen *et al*[67]., Studied that t he treatment of AIDS using combinations of antiretroviral drugs has highly reduced the HIV-1 related morbidity and mortality, provided that the plasma viral load can be maintained as low as possible by targeting the Nanoparticles

Anne S Ulrich[68]., reviewed that Liposomes are used as biocompatible carriers of drugs, peptides, proteins, plasmic DNA,antisense ligonucleotides or ribozymes, for pharmaceutical, cosmetic, and biochemical purposes. The enormous versatility in particle size and in the physical parameters of the lipids affords an attractive potential for constructing tailor-made vehicles for a wide range of applications.

Crispin R Dass[69]., reviewed that the Cationic liposomes are traditionally used for delivering macromolecules such as nucleic acids to mammalian and plant cells. This paper describes a novel simple and relatively inexpensive method for preparation of cationic liposomes usingan ethanol injection/pressure extrusion method. The study also evaluated the utility of a colorimetric method forquantification of cationic liposomes. Binding of erythrosine dye to cationic liposomes resulted in a shift of the absorptionmaximum of the dye from 528nm to 549nm in a buffer at pH 4.25, allowing quantification of these vesicles. Colourdevelopment was completed in

5 to 10 minutes at room temperature, with only 10% decrease in absorbance observed inthe following 2 hours. Divergent values were noted in the presence of interfering agents such as detergents and salts. Theerythrosine method is sensitive down to 0.20 μg/mL of cationic lipid and is linear to 3.13 μg/mL. The erythrosine dyemethod for quantitation of cationic liposomes is valuable for the field of liposome technology. In addition, a relatively to cationic liposomes from unbound molecules is presented. This method utilises a Ficoll-based gradient centrifugation method. Laboratory-formulated liposomes were just as efficient inbinding nucleic acids as commercially available types.

Tiwari sandip, B *et al*[70]., concluded that the thermosensitive liposomes encapsulated about 52% of the MTX. Comparison of the drugrelease profile at various temperatures revealed that maximum drug release (83%) occurred at 42°C compared to less than 5% release at 37°C.

Stefano Giovagnoliet *et al*[71]., studied that the unilamellar liposomes as new potential capreomycin sulfate (CS) delivery systems for future pulmonary targeting by aerosol administration. Dipalmitoylphosphatidylcholine, hydrogenated phosphatidylcholine, and distearoylphosphatidylcholine were used for liposome preparation. Peptide-membrane interaction was investigated by differential scanning calorimetry (DSC) and attenuated total internal reflection Fourier-transform infrared spectroscopy (ATIR-FTIR).

Anand Babu Dhanikula *et al*[72]., Alternative formulations of paclitaxel were developed in order to improve its aqueous solubility, and characterized in vitro. This water-soluble prodrug was encapsulated into polyethylene glycol coated liposomes optimized with saturated lipids,to overcome the physical instability associated with paclitaxel. Under in vitro testing, prodrug liposomes seem veryimpressive with release of only 45% of payload in 180 h. Further, chemical as well as physical stability studies haveshown that liposomes were stable without any signs of crystallization of paclitaxel

Mayank R Joshi *et al*[73]., studied that the stable liposomally entrapped budesonide (BUD) for a dry powder inhaler (DPI) formulation. BUD liposomes composed of egg phosphatidyl choline and cholesterol were prepared by lipid film

hydration technique and sonicated to have the desired size (< 5 μm). A rapid method was used for separation of free drug by centrifugation at a lower centrifugal force (G value).

Zhaohua Huang *et al*[74]., A series of thiocholesterol-based cationic lipids (TCL) has been designed and synthesized by theattachment of thiocholesterol to a cationic amine via a disulfide bond. TCL can be incorporated intoliposomes and used to package DNA into a lipoplex, thereby protecting it from DNase digestion.DNA is rapidly released from the complex in the presence of low concentrations of reducing agents.

Benjamin L Viglianti *et al*[75]., The purpose of this study was to determine if MnSO4/doxorubicin(DOX) loaded liposomes could be used for in vivo monitoring of liposome concentration distribution and drug release using MRI. In vitro results show that T1 shortening correlates with MnSO4 concentration. Using a temperature-sensitive liposome formulation, it was found that MnSO4 release significantly shortened T1. This feature, therefore, suggests that content release can also be measured with these MnSO4-loaded liposomes.

Jan A A M Kamps *et al*[76]., Human serum albumin (HSA) derivatized with cis-aconitic anhydride was covalently coupled to liposomes with a size of approximately 100 nm [polyaconitylated HSA (Aco-HSA) liposomes]. Within 30 min after injection into a rat, Aco-HSA liposomes were completely cleared from the blood and almost exclusively taken up by the liver, whereas in control liposomes 80% was still present in the blood at that time.

Yun- Long Tseng *et al*[77]., studied that the Unlike conventional liposomes, sterically stabilized liposomes, with their smaller volume of distribution and reduced clearance, preferentially convey encapsulated drugs into tumor sites.

Chun Man Lee *et al*[78]., An increased level of chondroitin sulfate (CS) expression on the cell surface is often associated with malignant transformation and the progression of tumor cells. In this study, CSs expressed on highly metastatic tumor cells were used as a target for the selective delivery of anticancerdrugs by polyethylene glycol (PEG)-coated liposomes that contained a newcationic lipid 3,5-dipentadecycloxybenzamidine hydrochloride (TRX-20).

Alberto A Gabizon *et al*[79]., at the turn of the 20th century, the German bacteriologist Paul Ehrlich coined the expression "magic bullets" in his search for chemotherapeutic agents with specific affinity for diseased tissues. Understanding target structure and function, developing drug delivery strategies to achieve controlled release, and targeting of drugs to specific tissues of the body have been a major focus of research in the last decades in an attempt to improve selectivity in cancer treatment.

Rajesh Kumar *et al*[80]., the objective of our study was to formulate and evaluate proliposomes in the form of enteric-coated beads using glyburide as a model drug. The beads were enteric coated with Eudragit L-100 by a fluidized bed coating process using triethyl citrate as plasticizer.

Rhoderick E Brown *et al*[81]., Recent cell biological studies suggest that sphingolipids andcholesterol may cluster in biomembranes to form raft-like

microdomains. Such lipid domains are postulated to function as platforms involved in the lateral sorting of certain proteins during their trafficking within cells as wellas during signal transduction events.

Mohamed Mahmoud Nounou *et al*[82]., Lyophilization increases the shelf-life of liposomes by preserving it in a dry form as lyophilized cake to be reconstituted with water immediately prior to administration. Aiming at increasing stability and availability of 5-Fluorouracil liposomal products, 5-Fluorouacil Stable Plurilamellar Vesicles were prepared. Freeze dried liposomal dispersions were prepared with or without cryoprotectants.

Xuemei Liang *et al*[83]., The micromechanical properties of pure and cholesterol modified egg yolk phosphatidylcholine (Egg PC) vesicles prepared by sonication were studied by atomic force microscopy (AFM) on mica surface.

Ana, I *et al*[84]., The success of the use of liposomes as drug carriers depends on both their formulation and the method of preparation. We have carried out a series of in vitro studies using different formulations and preparation methods, with the aim of obtaining a type of liposome which is efficient in the treatment of brucellosis.

Theresa Wiens *et al*[85]., A liposomal formulation capable of encapsulating 76 to 92% of the antimycobacterial drug ethambutol and showing prolonged in vitro release kinetics is described. In vitro efficacy is equivalent to that of the free drug, suggesting that encapsulation of ethambutol has the potential to shorten the current regimens for tuberculosis.

Cabral, E. C. M *et al*[86]., This work presents results of the preparation and characterization of small unilamellar liposomes for entrapping allergenic proteins extracted from the biomass of Dreschlera (Helminthosporium) monocer as cultivated by solid fermentation. Protein was entrapped by the dehydration-rehydration method, using lyophilization of preformed liposomes in order to prevent their degradation The reconstitution of lyophilizedliposomes by hydration, their capacity for entrapping allergenic proteins and their stability in plasma were analyzed.

Dimitrios G Fatouros *et al*[87]., In this study, we investigated non-sonicated arsonolipid-containing liposomes (arsonoliposomes) in terms of the influence of lipid composition ontheir stability, assessed as membrane integrity and physical stability [size].

Sharma G *et al*[88]., Solid tumors such as breast cancer have historically provided many challenges to anti-cancer therapy. Therapeutic hurdles to drug penetration in solid tumors include heterogeneous vascular supply and high interstitial pressures within tumor tissue,particularly in necrotic zones, lower pH and presence of leaky vasculature leading to reduced therapeutic response

Hidetoshi Arima *et al*[89]., In the present study, we examined tissue distribution and the antitumor effect of doxorubicin (DOX) after intravenous injection of the pegylated liposomes entrapping the DOX complex with g-cyclodextrin (g-CyD) (complex-in-liposome) inBALB/c mice bearing colon-26 tumor cells, compared with those of DOX solution, pegylated liposomes entrapping DOXalone (DOX-in-liposome), pegylated liposomes entrapping g-CyD (CyD-in-liposome) and the binary system of DOX-inliposome and CyD-in-liposome.

Masood A Khan *et al*[90]., Present study was performed to evaluate the efficacy, toxicity and pharmacokinetics of antifungal drug nystatin incorporated in immunomodulator tuftsin-bearing liposomes. In vitro toxicity of free nystatin and

nystatin incorporated in tuftsin-free ortuftsin-loaded liposomes was assessed by incubation of nystatin formulations with human erythrocytes.

Paraskevi Kallinteri *et al*[91]., The use of arsenic-containing compounds in cancer therapy is currently being reconsidered,after the recent approval of arsenic trioxide (Trisenox1) for the treatmentof relapsed promyelocytic leukemia (PML). In an attempt to prepare a carrier systemto minimize the toxicity of this drug, the aim of this study is to prepare andcharacterize liposomes encapsulating arsenic trioxide (ATO).

Ali Demir Sezer *et al*[92]., Liposomes are effectively used in the treatment of microbial infections. Higher cellular uptake has been reported when antibiotics are encapsulated in liposomes. In this study, enrofloxacin (ENF) was encapsulated in large unilamellar vesicles (LUVs) and the effects of formulation variables on the liposome characteristics were investigated. Liposomes were prepared using dry lipid film method.

Law, S. L *et al*[93]., Desmopressin-containing liposome formulations have been developed for intranasal administration previously. Positively charged liposomes were found to be an efficient delivery system for desmopressin. In this study, stability of the loaded desmopressin in positively charged liposomes was further investigated.Comparison of the stability of desmopressin in solution and liposomes was made.Degradation of desmopressin was shown to follow a pseudo-first-order reaction.

Koromil, A *et al*[94]., A method to correct stent related complications non-invasively, is the local delivery oftherapeutic agents. Different drugs have been delivered on stents, after being eitherdispersed or encapsulated in polymeric materials, and placed on stents to form drugeluting-stents (DE-stents). Investigation of possibility to cover polymer - coated metallic stents, with liposomal drugs, for preparation of novel DE-liposome-coated-stents,has been initiated few years ago.

Jringjai Thongborisute *et al*[95]., To optimize the properties of chitosan-coated liposomes for oral administration of peptide drugs, we examined the effect of type of chitosan and the structure of liposomal systems on the mucoadhesiveness of liposomes and resultant pharmacological effects of the liposomal peptide drug. A

low-molecular weight chitosan (LCS) and a high-molecular weight chitosan (CS) were used as coating polymers of liposomes containing elcatonin (eCT). The muco-penetrative behaviors across the mucous gellayer covering the intestinal epithelial cells and the pharmacological effect after intragastric administration were determined in rats.

Minakshi Garg ***et al*[96]*.,*** In order to target liposomes to the lectin receptors present on macrophages, galactosylated liposomes were prepared and characterized in vitro. O-palmitoylgalactose (OPG) for liposomal coating was synthesized by esterification of galactose with palmitoyl chloride. The galactose binding Ricinus communis lectin was employed as a model system for the determination of in vitro ligand binding capacity. Cellular drug uptake studies were performed using alveolar macrophages.

Jia-You Fang ***et al*[97]*.,*** Tea polyphenols, including (þ)-catechin, (2)-epicatechin, and (2)-epigallocatechin-3-gallate (EGCG), have been shown topossess potent antioxidant and anticancer activities. The aim of this study was to evaluate the possibility of using liposomes for the local delivery, including skin and tumor deposition, of these polyphenols. Liposomes containing egg phosphatidylcholine, cholesterol, or anionic species were prepared by a solvent evaporation method and then were subjected to a probe sonicator. The size, zeta potential and entrapment efficiency of these liposomal formulations were determined to provide correlations with results from a subsequent in vivo study.

Barbara Ruozi ***et al*[98]*.,*** Liposomes loaded with ketorolac tromethamine salt were prepared by using a thinlayer evaporation method. The physical properties of liposomes were studied by usingatomic force microscopy (AFM) and transmission electron microscopy (TEM). Therelationship between lipid composition, encapsulation efficiency, vesicle size, and the release of ketorolac tromethamine-loaded liposomes was studied.

Tareq Taha Jubeha ***et al*[99]*.,*** Superoxide dismutase (SOD), 4-amino tempol (tempamine, denoted as TMN) and catalase were encapsulated into negatively charged liposomes. The activity of the antioxidants against dinitrobenzenesulfonic

acid (DNBS) induced colitis was tested in the rat and compared to the anti-inflammatory activity of the native enzymes and free TMN. as ulcerative colitis.

Zelihag L Deg *et al*[100]., Insulin is a polypeptide drug and it is degraded by gastrointestinal enzymes, therefore, it cannot be used via oral route readily. There are only parenteral forms available in the market. The aim of this study was to investigate the effect of rectal and vaginaladministration of various insulin gel formulations on the blood glucose level as alternative routes in rabbits. Chitosan gel (CHgel) was used as a carrier; the penetration enhancing effect of sodium taurocholate and dimethyl-b-cyclodextrin (DM-bCD)was also investigated.

Thongborisute, J *et al*[101]., The objectives of this study were to observe the penetrative and mucoadhesive behaviorof polymer-coated liposomes into the intestinal mucosa of rats. Chitosan (CS) andnegatively charged liposomes were chosen as model polymer-coated liposomes. Inorder to observe their behavior, chitosan was labeled with Fluorescence Isothiocyanate(FITC) via chemical reaction at the isothiocyanate group of FITC and the primaryamino group of chitosan; the liposomes (Lips) were marked by incorporation of Dil into the liposomal formulation.

Loan Honey Wel-Nguyen, P *et al*[102]., The main problem in delivery of drugs across the skin is the barrier function of the skin, which is located in the outermost layer of the skin, the stratum corneum. The stratum corneum consists of corneocytes surrounded by lipid layers, the so-called lipid lamellae. When applying drugs onto the skin, the major penetration pathway is the tortuous intercellularroute along the lipid lamellae. In order to increase the number of drugs administeredvia the transdermal route, novel drug delivery systems have to be designed. Amongthese systems are iontophoresis, electroporation, microneedles, and vesicular systems.

Vandana Soni *et al*[103]., Diseases and disorders of the brain are extremely difficult to treat pharmacologically because most drugs are unable to passacross the blood–brain barriers. In the present work, a transferrin-coupled liposomal system for brain delivery of 5-florouracil has been investigated.5-florouracil and 99mTc-DTPA bearing non-coupled liposomes were prepared by cast film method, which were coupledwith the transferrin by incubating these liposomes with transferrin in the

presence of the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in saline phosphate buffer (pH 7.4). These liposomal systems were characterized for vesicle size, percent drug entrapment, and in vitro drug release.

Carl R Alving[104]., was studied naturally occurring antibodies to phospholipids and cholesterol are widespread; they occur commonly during the course of acute infections; they are not causally related to the anti-phospholipid syndrome; they have been associated with other clinical entitiesonly as an epiphenomenon; and they have not been implicated as causing any clinicalsyndrome or disease

Theresa M Allen *et al*[105]., The use of targeted liposomes is recognized as a promising strategy for improving the selective targeting of drugs to diseased tissues in vivo, leading to reductions in drug toxicity and improvements in therapeutic outcomes. A large variety of targeting molecules have been attached to the surface of liposomes to date, using a variety of coupling methods. If targeted liposomes are to be useful in clinical applications

Lian Zheng et *al*[106]., An important aspect of vaccine development involves delivery of antigens to antigen-presenting cells for the induction of potent antigen-specific T lymphocyte responses. We investigated the effect of a cationic liposome, lipofectin, on delivery of whole proteins to human dendritic cells (DCs) derived from blood mononuclear cellsby culture in interleukin 4 and granulocyte-monocyte colony-stimulating factor for stimulation of human immunodeficiencyvirus type 1 (HIV-1)-specific memory cytotoxic T lymphocyte (CTL) responses.

Holger Grohganz *et al*[107]., The purpose of this research was to adapt a colorimetric, phospholipase D-based serum-phospholipid assay for the quantification of phosphatidylcholine (PC) in liposomes using a microtitre plate reader. PC from natural egg PC lipo-somes was quantified reliably. In contrast, poor sensitivity was found for liposomes composed of saturated PCs (di-palmitoyl-phosphatidylcholine [DPPC], hydrogenated egg PC). Triton X-100 was then added to the liposomes fol-lowed by heating above the phase transition temperature..

Lars Ingebrigtsen *et al*[108]., In this study, small liposomes obtained by high-pressure homogenization were fractionated according to their particle sizes by size exclusion chromatography (SEC). The subfractions were analyzed by photon

correlation spectroscopy (PCS) as well as enzymatic phosphatidylcholine (PC) assay for their particle sizes and lipid contents, respectively.

Sanjay K Jain et *al*[109]., The aim of the present study was to design a depot delivery system of acyclovir sodium using multivesicular liposomes (MVLs) to overcome the limitations of conventional therapiesand to investigate its in vivo effectiveness for sustaineddelivery. MVLs of acyclovir were prepared by the reverse phase evaporation method. The loading efficiency of the MVLs (45%-82%) was found to be 3 to 6 times higher than conventional multilamellar vesicles (MLVs). The in vitrorelease of acyclovir from MVL formulations was found to be in a sustained manner and only 70% of drug was released in 96 hours, whereas conventional MLVs released 80% of drug in 16 hours. Following intradermal administration to Wistar rats, the MVL formulations showed effective plasma concentration for 48 hours compared with MLVs and free drug solution (12-16 hours).

Jean-Paul Leonetti *et al*[110]., Mouse L929 cells were incubated with antibody-targeted liposomes containing oligodeoxyribonucleotides(oligomers). When the oligomer was a 15-mer complementaryto the 5'-end region of the mRNA encoding the N protein of vesicular stomatitis virus, the cells became less permissive formultiplication of that virus; >95% reduction of viral multiplication was achieved. Protection was not seen for "empty" liposomes, liposomes containing a random oligomer sequence, or liposomes containing a sequence complementary to the 5'end of c-myc protooncogene mRNA targeted by the sameantibody, nor was it seen when the liposomes containing the N-protein antisense oligomer were targeted by an antibody that does not bind to L929 cells.

Martin, B *et al*[111]., Synthetic gene delivery vectors are gaining increasing importance in gene therapy as an alternative torecombinant viruses. Among the various types of non-viral vectors, cationic lipids are especially attractive as they can be prepared with relative ease and extensively characterized.

Fenart, L *et al*[112]., A cell culture model of the blood-brain barrier (BBB) consisting of a co culture of bovine brain capillary endothelial cells and rat astrocytes has been used to examine the ability of 60-nmnanoparticles with different physicochemical characteristics tocross the BBB. . Lipid-coated nanoparticles

werenontoxic toward BBB integrity, and crossed the BBB by transcytosiswithout any degradation. Furthermore, a 27-fold increasein albumin transport was observed when albumin hadpreviously been loaded in the cationic lipid-coated nanoparticles.

Milan Stuchlík et *al*[113]. With an increasing number of lipophilic drugs under development, homolipids and heterolipids have gainedrenewed interests as excipients for oral drug delivery systems. Oral administration has many advantages for chronic drug therapy. It is relatively safe, convenient for the patient and allows self administration.

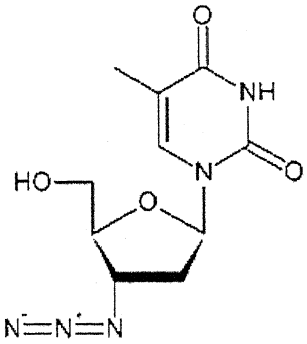
Chapter-4

Drug and Lipid Profile

DRUG AND LIPID PROFILE

4.1 DRUG PROFILE (ZIDOVUDINE) :

Chemical structure:



IUPAC name	:	1-[(2*R*,4*S*,5*S*)- 4-azido-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-pyrimidine-2,4-dione
Formula	:	$C_{10}H_{13}N_5O_4$
Mol. Mass	:	267.242 g/mol
Bioavailability	:	Near complete absorption, following first-pass metabolism systemic availability 65% (range 52 to 75%)
Protein binding	:	30-38%
Metabolism	:	Hepatic
Half life	:	0.5-3 hours
Excretion	:	Renal
Color	:	White to off-white
Odor	:	Odor less
Appearance	:	Crystalline solid

Zidovudine is an antiretroviral drug, the first approved for treatment of HIV. Zidovudinwas the first drug approved for the treatment of AIDS and HIV infection[114]. It was originally intended to treat cancer, but failed to show efficacy and had an unacceptably high side effect profile. After showing that this drug was an effective agent against HIV *in vitro*, the team conducted the initial clinical trial that

provided evidence that it could increase CD4 counts in AIDS patients. In AZT subsequently conducted clinical trials could prolong the life of patients with AIDS.

The Food and Drug Administration(FDA) approved the drug for use against HIV, AIDS, on March 20, 1987, and then as a preventive treatment in 1990. It was initially administered in much higher dosages than today, typically 400 mg every four hours (even at night). However, the unavailability at that time of alternatives to treat AIDS affected the risk/benefit ratio, with the certain toxicity of HIV infection outweighing the risk of drug toxicity. One of AZT's side effects includes anemia, a common complaint in early clinical trials[115,116].

Pharmacology:

Zidovudine is an inhibitor of the invitro replication of some retroviresus incluiding HIV. Retroviresuscontains reverse transcriptase, an essential enzyme for the life cycle of the retrovirus[117].

Mechanism of action:

Zidovudine is converted to monophosphate, diphosphate, triphosphate derivativesby host cell thymidine kinase. Zidovudinetriphosphate is the active which competes with thymidine 5- triphosphate to binding to HIV RT. The HIV by mistake incorporates zidovudine triphosphate in viral replication.consequently zidovudine acts as a chain terminater in the DNA synthesis resulting in inhibition of HIV replication[118,119].

Antimicrobial activity:

Zidovudine has effect on different types of bacteria -

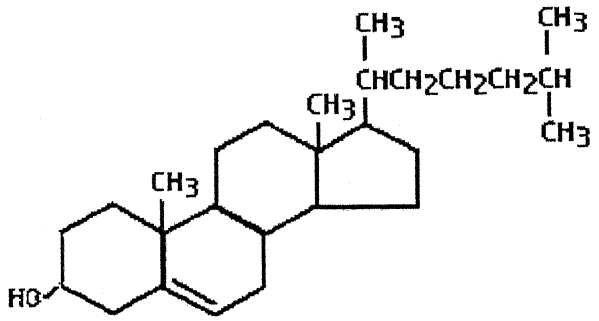
(a) Gram negative bacteria- Salmonelle typhimurium, Esherichia ccoli, Klebsela pneumoniae.

(b) Gram positive bacteria- Staphylococcus aureus, Staphylococcus pyogenes, Pseudomonas aaeurinosa, Mycobacterium tuberculosis[120].

4.2 LIPID PROFILE:

4.2.1 Cholesterol :

Structure :



Formula : $C_{27}H_{46}O$

IUPAC name : (10S,13R)-10,13-dimethyl-17-(6-methyl heptane-2-yl)-2,3,4,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopent [a], phenanthren-3-ol.

Molecular weight : 386.7

Appearance : White crystalline color

Melting point : 147°C-150°C

Solubility : Insoluble in water, Soluble in organic solvents

Storage : In well closed container, protect from light

General Description :

This molecule is composed of three regions a hydrocarbon tail, a ring structure region with 4 hydrocarbon rings, and a hydroxyl group. The hydroxyl (OH) group is polar, which makes it soluble in water. This small 2-atom structure makes cholesterol an alcohol. The alcohol that we drink, ethanol, is a much smaller alcohol that also has a hydroxyl group (C_2H_5OH).

The 4-ring region of cholesterol is the signature of all steroid hormones (such as testosterone and estrogen). All steroids are made from cholesterol. The rings are called hydrocarbon ring because each corner of the ring is composed of a carbon atom, with two hydrogen atoms extending off the ring. The combination of the steroid

ring structure and the hydroxyl (alcohol) group classifies cholesterol as a "sterol." Cholesterol is the animal sterol. Plants only make trace amounts of cholesterol, but make other sterols in larger amounts. The last region is the hydrocarbon tail like the steroid ring region; this region is composed of carbon and hydrogen atoms. Both the ring region and tail region are non-polar, which means they dissolve in fatty and oily substances but will not mix with water. Because cholesterol contains both a water-soluble region and a fat-soluble region, it is called Amphipathic.

Sources of Cholesterol:

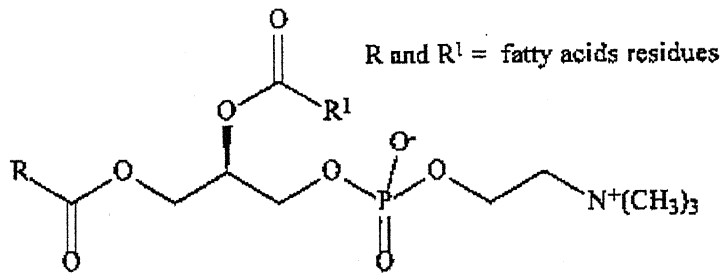
Cholesterol is found in animal fats: all food containing animal fats contains cholesterol; food not containing animal fats contains no cholesterol or negligible amounts. Major dietary sources of cholesterol include eggs, beef and poultry. Plants have trace amounts of cholesterol, so even a vegetarian diet, which includes no animal foods, has traces of cholesterol. However, the amounts are very small. For example, the amount of cholesterol in one egg is approximately equal to the amount in 9.6 liters (19.57 pounds) of pure peanut oil. Plant products (e.g. peanut), also contain cholesterol-like compounds, phytosterol, which are suggested to help lower serum cholesterol.

Function :

Cholesterol is required to build and maintain cell membranes it regulates membrane fluidity over a wider range of temperatures. The hydroxyl group on cholesterol interacts with the phosphate head of the membrane, while the bulky steroid and the hydrocarbon chain is embedded in the membrane. Some research indicates that cholesterol may act as an antioxidant. Cholesterol also aids in the manufacture of bile (which stored in the gallbladder and helps digest fats), and is also important for the metabolism offat soluble vitamins, including vitamins A, D, E and K. It is the major precursor for the synthesis of vitamin D and of the various steroid hormones (which include cortisol and aldosterone in the adrenal glands, and the sex hormones progesterone the various estrogens testosterone and derivatives).

4.2.2 Phosphotidylcholine :

Chemical structure :



Synonyms : L-a-Lecithin, 3-Sn-phophotidylcholine

Molecular Weight : 776.12

Solubility : Soluble at room temperature in chloroform, ethanol and Hexane containing 3% ethanol.

Storage temperature : 0-4°C

General description:

Phosphatidylcholine is a phospholipid that is a major constituent of cell membranes. Phosphatidylcholine is also known as 1, 2-diacyl-:ussn:ue-glycero-3-phosphocholine.

The term lecithin itself has different meanings when used in chemistry and biochemistry than when used commercially. Chemically, lecithin is phosphatidylcholine. Commercially, it refers to a natural mixture of neutral and polar lipids. Phosphatidylcholine, which is a polar lipid, is present in commercial lecithin in concentrations of 20 to 90%. Most of the commercial lecithin products contain about 20% phosphatidylcholine. Lecithins containing phosphatidylcholine are produced from vegetable, animal and microbial sources, but mainly from vegetable sources. Soybean, sunflower and rapeseed are the major plant sources of commercial lecithin. Soybean is the most common source. Plant lecithins are considered to be GRAS (generally regarded as safe). Egg yolk lecithin is not a major source of lecithin in

nutritional supplements. Eggs themselves naturally contain from 68 to 72% phosphatidylcholine, while soya contains from 20 to 22% phosphatidylcholine.

The fatty acid makeup's of phosphatidylcholine from plant and animal sources differ. Saturated fatty acids, such as palmitic and stearic, make up 19 to 24% of Soya lecithin; the monounsaturated oleic acid contributes 9 to 11%; linoleic acid provides 56 to 60%; and alpha-linolenic acid makes up 6 to 9%. In egg yolk lecithin, the saturated fatty acids, palmitic and stearic, make up 41 to 46% of egg lecithin. Unsaturated fatty acids are mainly bound to the second or middle carbon of glycerol. Choline comprises about 15% of the weight of phosphatidylcholine.

Pharmacology:

Phosphatidylcholine may have hepatoprotective activity. Phosphatidylcholine is important for normal cellular membrane composition and repair. Phosphatidylcholine is also the major delivery form of the essential nutrient choline. Choline itself is a precursor in the synthesis of the neurotransmitter acetylcholine, the methyl donor betaine and phospholipids, including phosphatidylcholine and sphingomyelin among others. Phosphatidylcholine is involved in the hepatic export of very-low-density lipoproteins.

Mechanism of action:

Phosphatidylcholine's role in the maintenance of cell-membrane integrity is vital to all of the basic biological processes. These are: information flow that occurs within cells from DNA to RNA to proteins; the formation of cellular energy and intracellular communication or signal transduction. Phosphatidylcholine, particularly phosphatidylcholine rich in polyunsaturated fatty acids, has a marked fluidizing effect on cellular membranes. Decreased cell-membrane fluidization and breakdown of cell-membrane integrity, as well as impairment of cell-membrane repair mechanisms, are associated with a number of disorders, including liver disease, neurological diseases, various cancers and cell death.

Pharmacokinetics:

Phosphatidylcholine is absorbed into the mucosal cells of the small intestine, mainly in the duodenum and upper jejunum, following some digestion by the

pancreatic enzyme phospholipase, producing lysophosphatidylcholine (lysolecithin). Reacylation of lysolecithin takes place in the intestinal mucosal cells, reforming phosphatidylcholine, which is then transported by the lymphatics in the form of chylomicrons to the blood. Phosphatidylcholine is transported in the blood in various lipoprotein particles, including very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL); it is then distributed to the various tissues of the body. Some phosphatidylcholine is incorporated into cell membranes. Phosphatidylcholine is also metabolized to choline, fatty acids and glycerol. The fatty acids and glycerol either get oxidized to produce energy or become involved in lipogenesis. Choline is a precursor of acetylcholine. Serum choline levels peak between 2 to 6 hours after oral intake.

Indication and usage:

Phosphatidylcholine may be indicated to help restore liver function in a number of disorders, including alcoholic fibrosis, and possibly viral hepatitis. It may also be indicated for the treatment of some manic conditions. There is some evidence that Phosphatidylcholine may be useful in the management of Alzheimer's disease and some other cognitive disorders. A possible future role in cancer therapy is also suggested by recent research.

Chapter-5

Materials and Instrument Used

Chapter-5

MATERIALS & INSTRUMENTS

5.1 MATERIALS:

5.1.1 Drug:

- **Zidovudine** : Gift sample from Alkem laboratories Pvt Ltd, Raigad.

5.1.2 Lipids:

- **Soya Lecithin** : Himedia laboratories, Mumbai.
- **Cholesterol** : Qualigens fine chemicals, Mumbai.

5.1.3 Polymers:

- **Carbopol-974** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **Carbopol-940** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **Carbopol-971** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **HPMC K4 100** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **HPMC K4 100** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **HPMC K4 100** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.
- **Chitosan** : Gifted by Central Fisheries, Cochin
- **Polaxamer** : Gifted by Colorcon Asia Pvt Ltd, Verna, Goa.

5.1.4 Chemicals:

- **Ethanol** : Qualigens fine chemicals, Mumbai
- **Chloroform** : Merck, Mumbai.
- **Octanol** : Loba Chemicals, Mumbai.
- **Triton X-100** : Loba fine chemical. Mumbai.

- **Potassium di hydrogen Phosphate** : CDH , India
- **Sodiumdihydrogen phosphate** : CDH, India
- **Sodium hydroxide** : CDH, India
- **Potassium hydroxide** : CDH, India
- **Lactic acid** : CDH, India
- **Acetic acid** : CDH,India

5.2 INSTRUMENTS USED:

- **U-V spectrometer** : Model 1700-E Shimadzu, Japan.
- **Centrifuge** : CPR-24, Remi equipments, India.
- **Centrifuge** : Zenith eng, INDIA.
- **Rotary Vacuum evaporator:** CRYOCHILLER, 102/102, Italy.
- **Electronic balance** : Roy electronics, INDIA.
- **Single pan balance** : 200/A, Deluxe sigma, INDIA
- **Vortex shaker** : Jyoti scientific industry, INDIA.
- **PH meter** : VSI-IB, VSI electronics, INDIA.
- **FTIR** : Spectrophotometers, 8201PC (4000-400/cm), Shimadzu, Japan.
- **Deep Freezer** : Operan, Korea
- **Bucci type rotary evaporator:** Zenith eng, INDIA.
- **Transition electron Microscopy (TEM)** : FEI,Nethrelands

Others

- **Dialysis membrane** : Hi-Media, India

Chapter-6

Experimental

EXPERIMENTAL

6.1 STANDARD CURVE PREPARATION:

6.1.1 Spectrophotometric analysis of Zidovudine:

The content of zidovudine was estimated by spectrophotometric method. Pure zidovudine was taken and the solutions were prepared by using saline solution as solvent, viz. 1mcg/ml, 2mcg/ml, 3mcg/ml, 4mcg/ml, 5mcg/ml, 6mcg/ml. The absorbance was measured by using U-V spectrophotometer at λ max 267 nm using saline as blank.

Straight line was obtained for a drug concentration of 1mcg/ml to 6mcg/ml. The drug obeys the Beers law between 1-6 mcg/ml concentrations.

Table-01: Standard curve of Zidovudine in water.

S.No.	Concentration (μg)	Absorbance (nm)
1.	1	0.051
2.	2	0.098
3.	3	0.155
4.	4	0.2
5.	5	0.253
6.	6	0.296

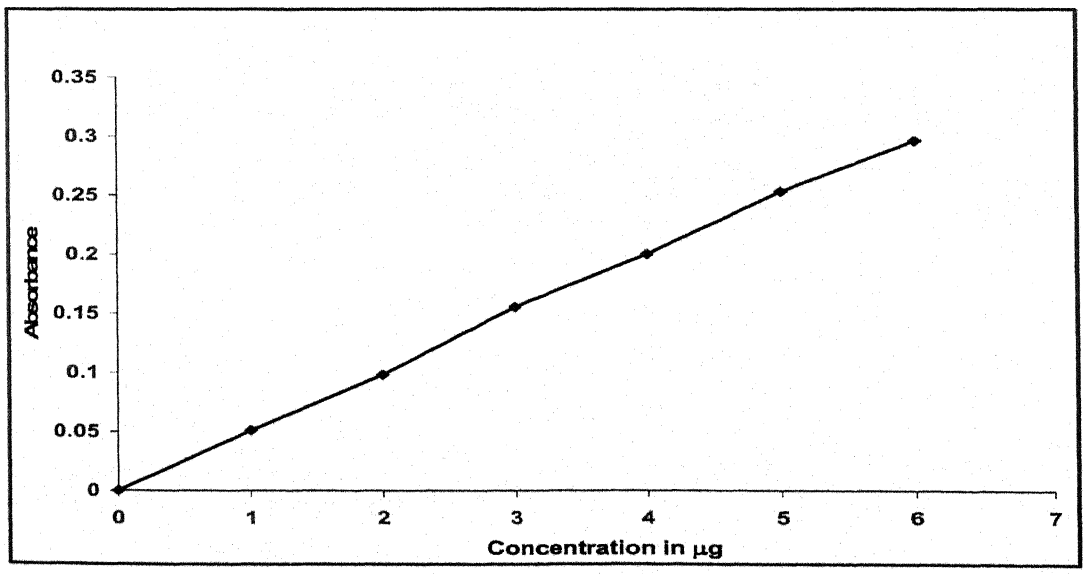


Figure-16 Standard curve of Zidovudine in water.

6.1.2 Partition coefficient (Distribution coefficient):

The partition coefficient is defined as the ratio of unionized drug distributed between the organic phase and aqueous phase at equilibrium. For a drug delivery system, Lipophillic/hydrophilic balance has shown to be a contributing factor for the rate and extent of drug absorption. Partition coefficient provides a means of characterizing Lipophillic/hydrophilic nature of drug. The measurement of drug lipophilicity and indication of its ability to cross the lipoidal cell membrane is the oil/water partition coefficient in system such as octanol/water.

Procedure:

The partition coefficient of drug (Zidovudine) was determined in solvent system- Octanol/Distilled water. Accurately weighed quantity of drug (10 mg) taken in one stoppered glass vial containing 5 ml of octanol, 5ml of distilled water was added to the vial. Then the glass vial was kept to equilibrate by shaking in vortex mechanical shaker for 6 hours and after shaking, the vial containing materials were transferred into a separating funnel, kept over night at room temperature for equilibrium.. After appropriate dilutions, the aqueous phase was analyzed for zidovudine against reagent blank solution using shimadzu-1700 E U-V spectrometer. The drug concentration in octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of drug added to the vials. The partition coefficient value P was calculated by the following equation.

$$P_{o/w} = (C_{organic} / C_{aqueous})$$

$$P_{w/o} = (C_{aqueous} / C_{organic})$$

Table-02: Partition coefficient (PC) of Zidovudine in Octanol (O), Distilled water (W)

S. No	PC in O/DW	PC in DW /O
1.	0.253	0.250

Infrared spectrum study:

Infrared spectroscopy is the study of interaction of infrared light with matter. The fundamental measurement obtained in infrared spectroscopy is an infrared spectrum, which is a measurement of infrared intensity versus wavelength ort wave number of light. As instrument used to obtained and infrared spectrum is called an infrared spectrometer. There are several kind of spectrometers used to obtained infrared spectra. The most prevalent type of spectrometer is called as Fourier transform infrared spectrometer (FTIR). Infrared spectroscopy is sensitive to the presence of chemical functional groups in a sample. Functional groups are a structural fragment within a molecule. Functional groups often have chemical properties that are the same from molecule to molecule.

The most powerful aspect of infrared spectroscopy is that it allows identifying unknowns. Once the wave number position of the band of a functional group is knows, this information can be used to identify that functional group in many samples.

A second use of infrared spectra is in confirming identities. Identities involve comparing the spectra of two samples to each other to determine whether the samples have the same composition.

Finally, the peak intensity in an infrared spectrum is proportional to concentration, so infrared spectra can be used to measure concentration as well. In the given spectra peaks are compared with standard peaks and concluded that there was no any interaction between drug and polymers.

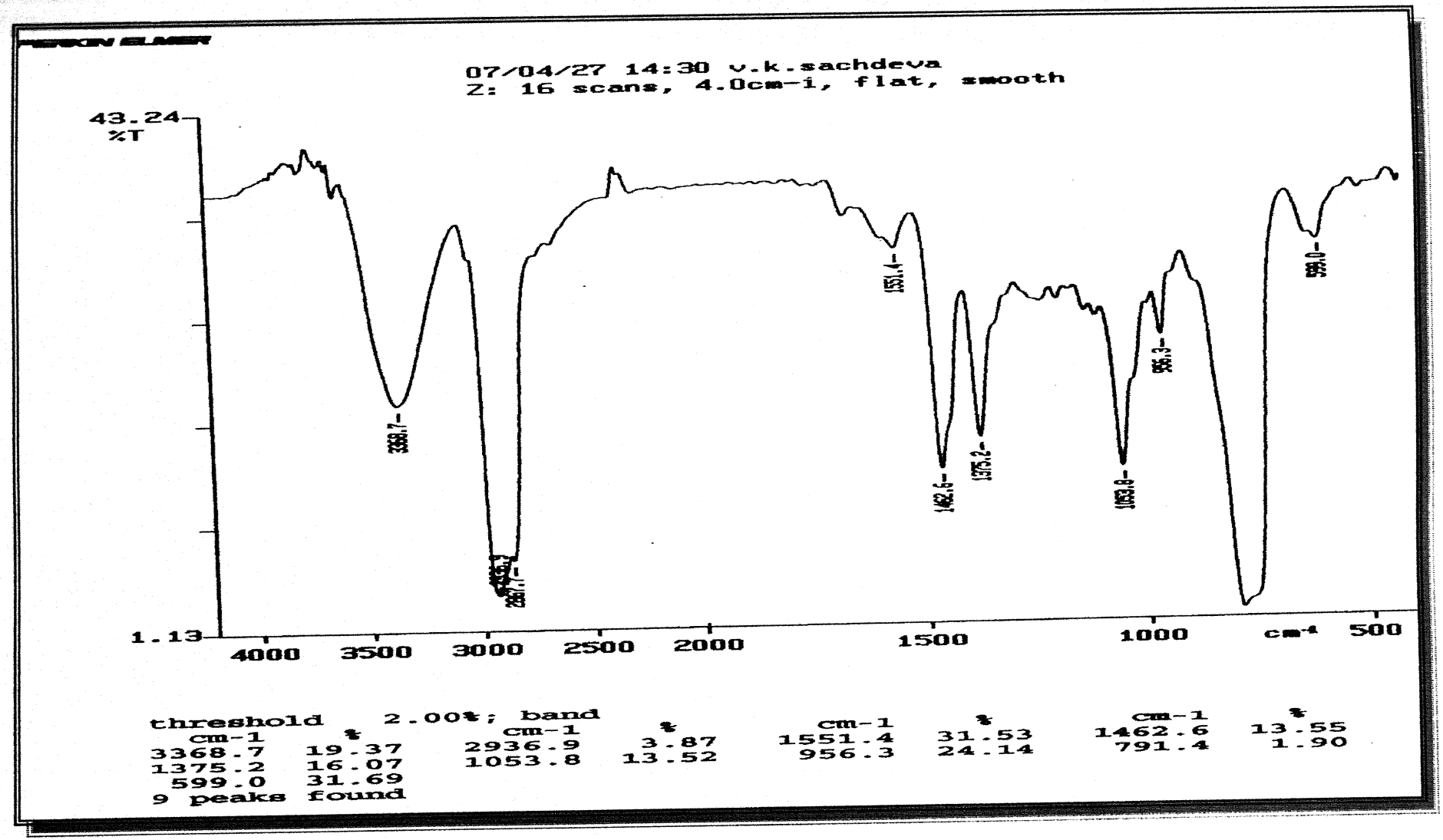


Figure-17: FTIR of the Cholesterol

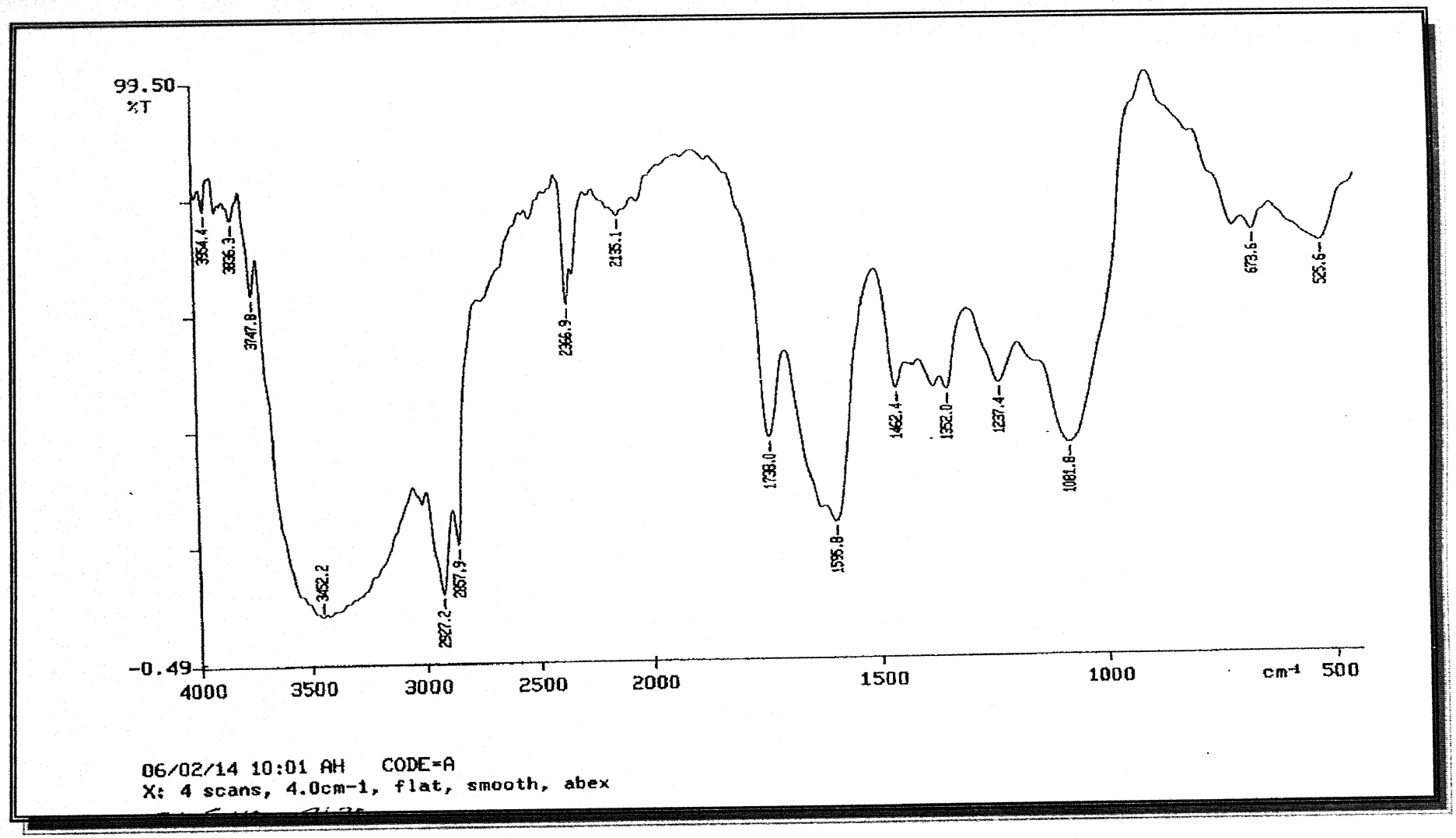


Figure-18: FTIR of the Phophotidycholine

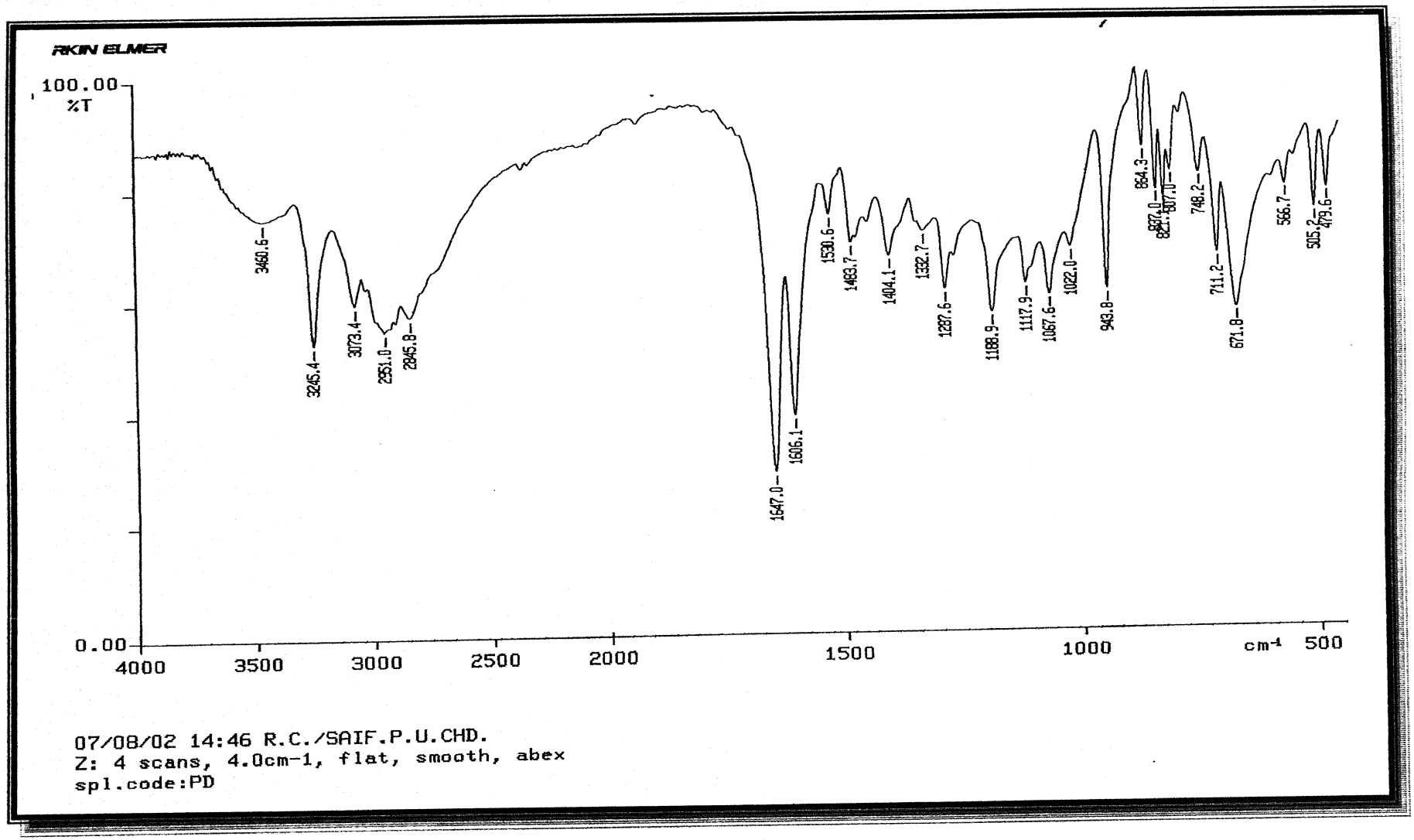


Figure-19: FTIR of the Zidovudine

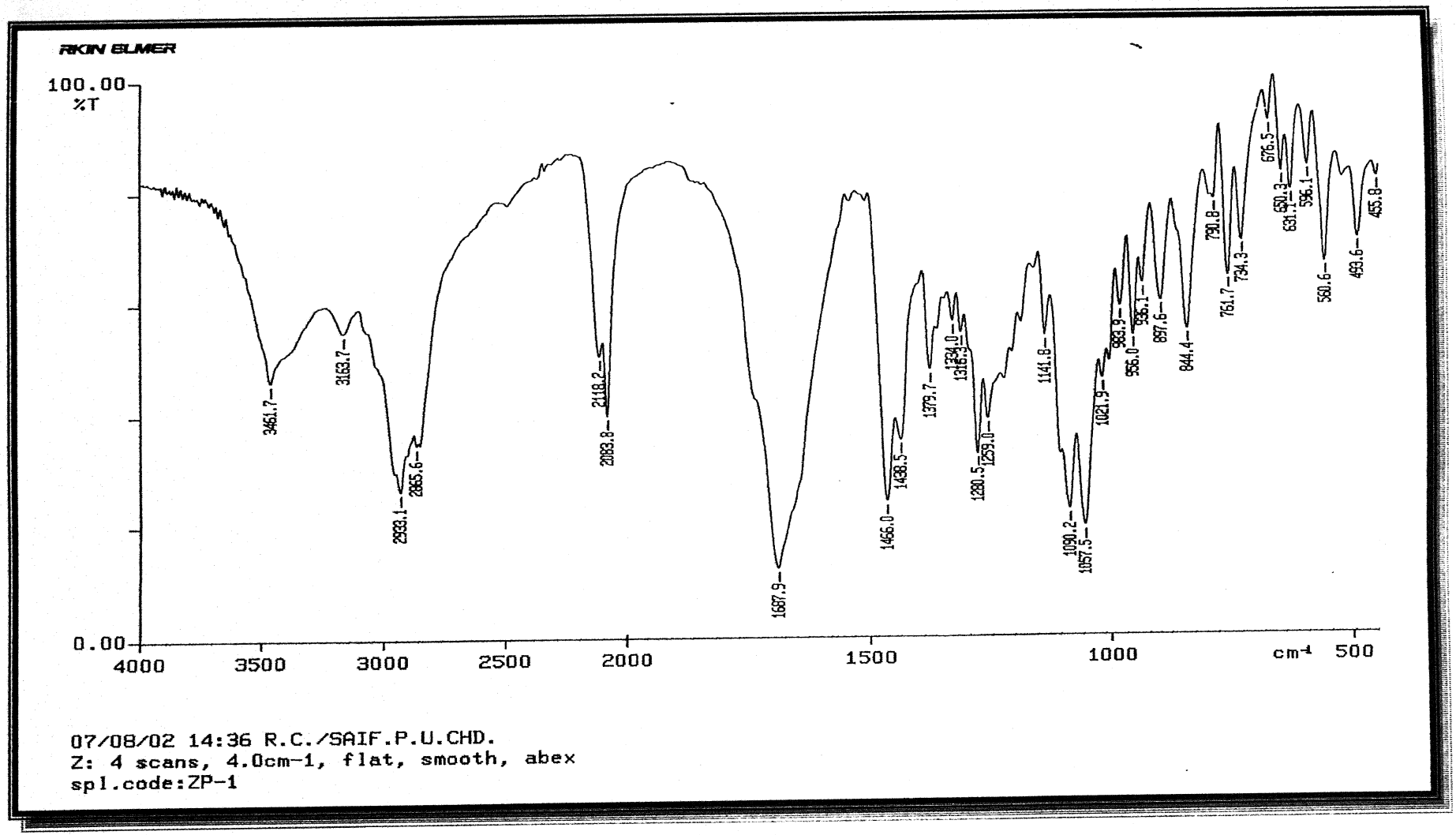


Figure-20 : FTIR of the Zidovodine + Phosphotidylcholine + Cholesterol

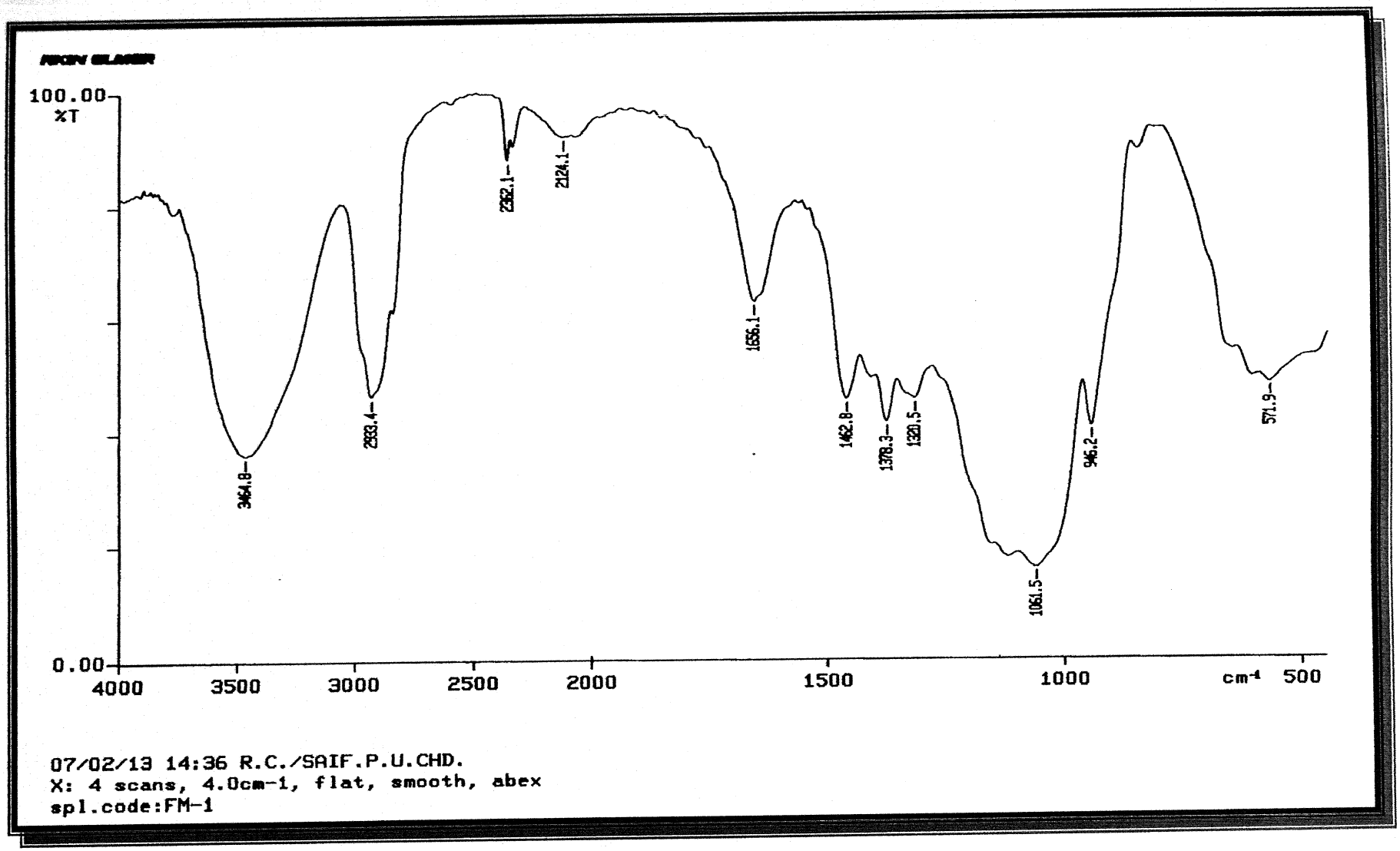


Figure-21: FTIR of the HPMC

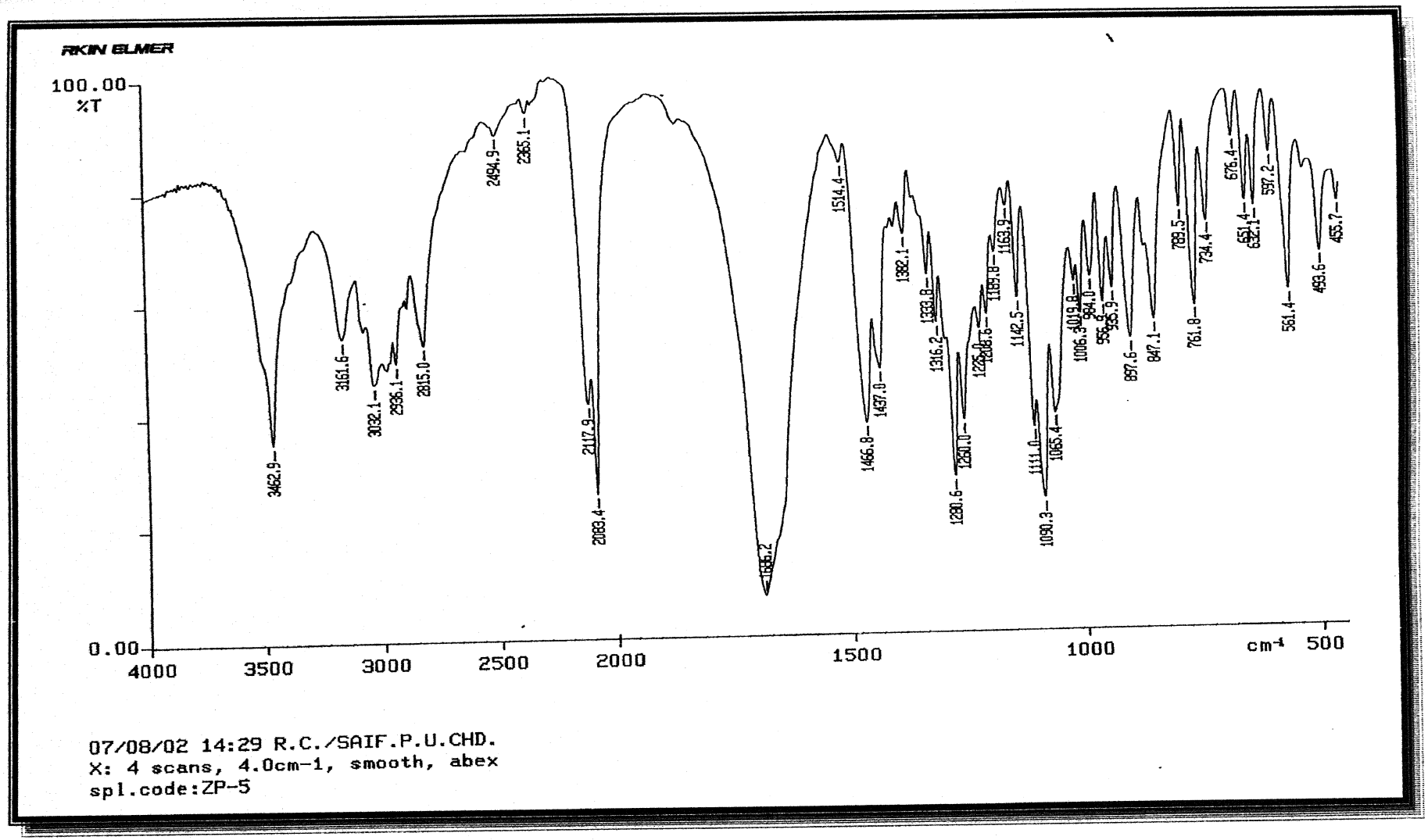


Figure-22: FTIR of the Zidovodine + HPMC

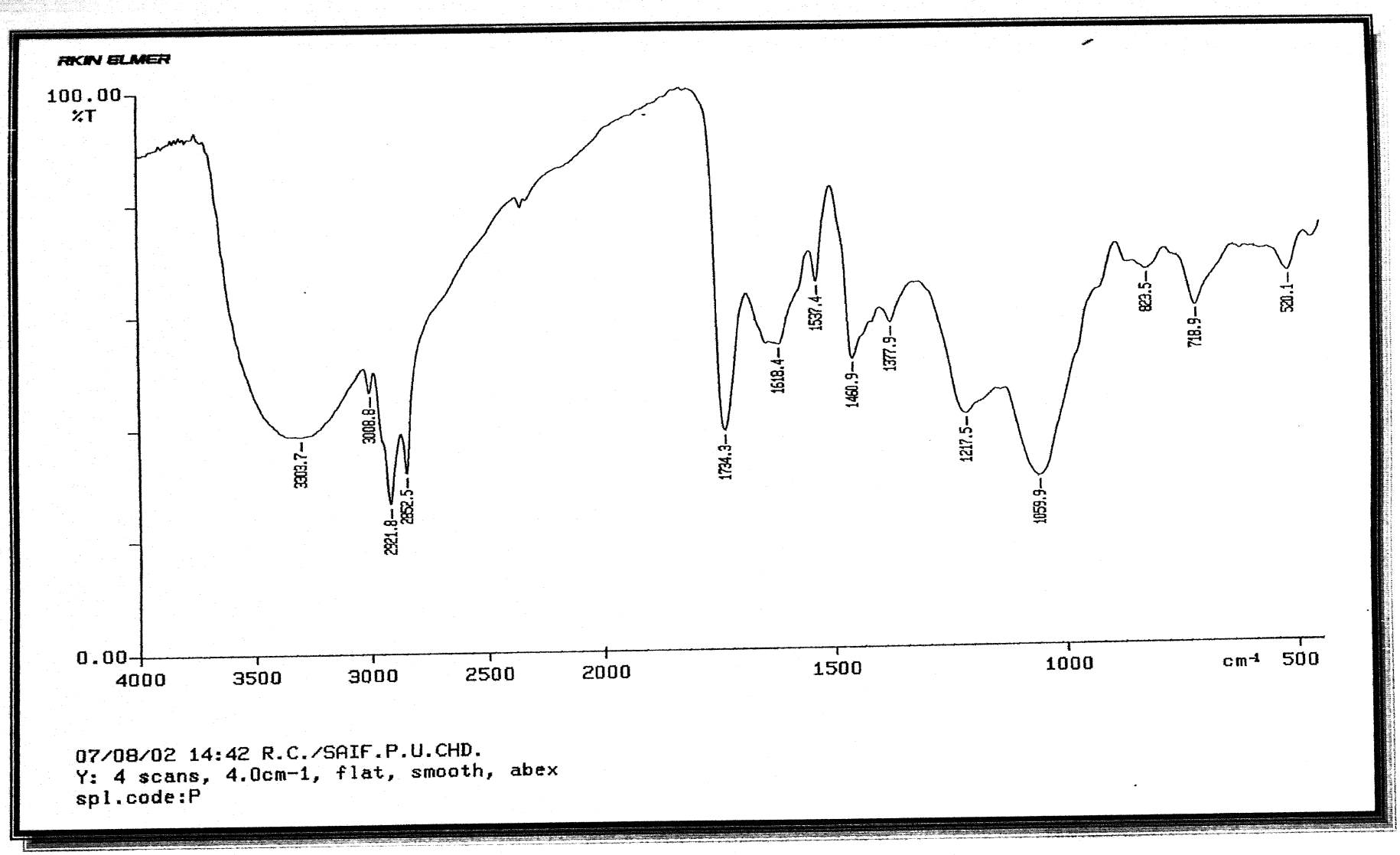


Figure-23: FTIR of the Polaxamar

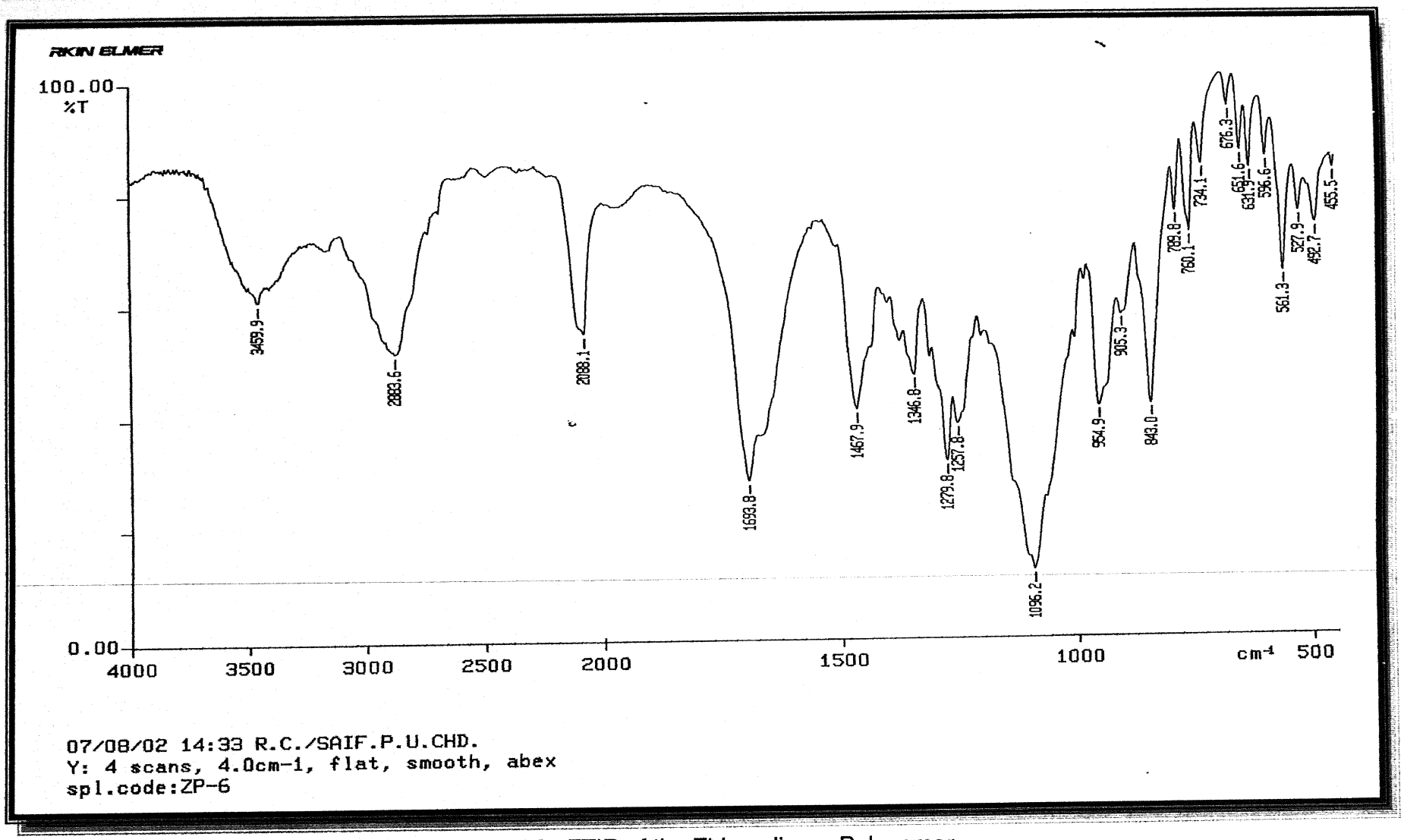


Figure-24: FTIR of the Zidovodine + Polaxamar

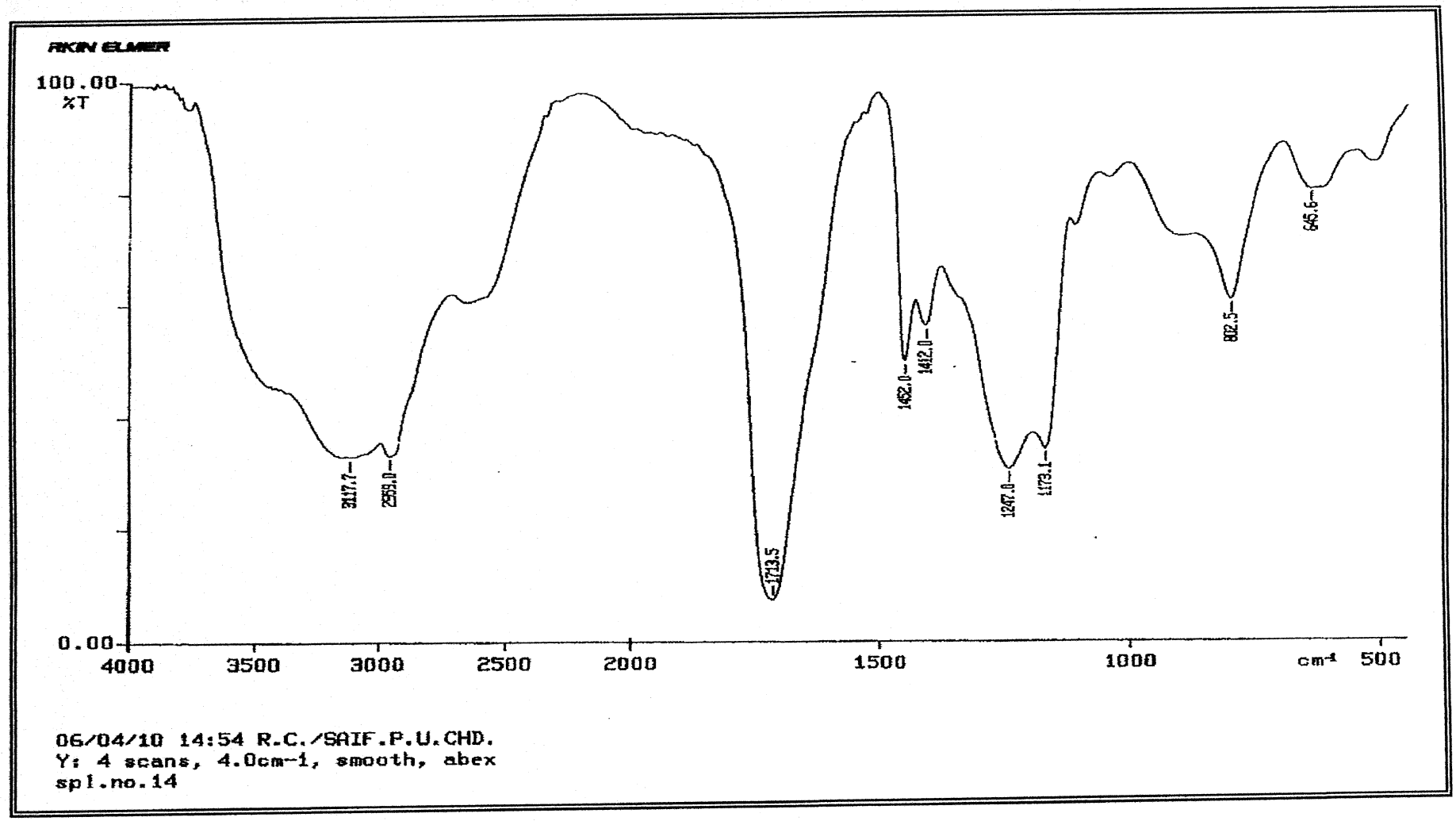


Figure-25: FTIR of the Carbopol

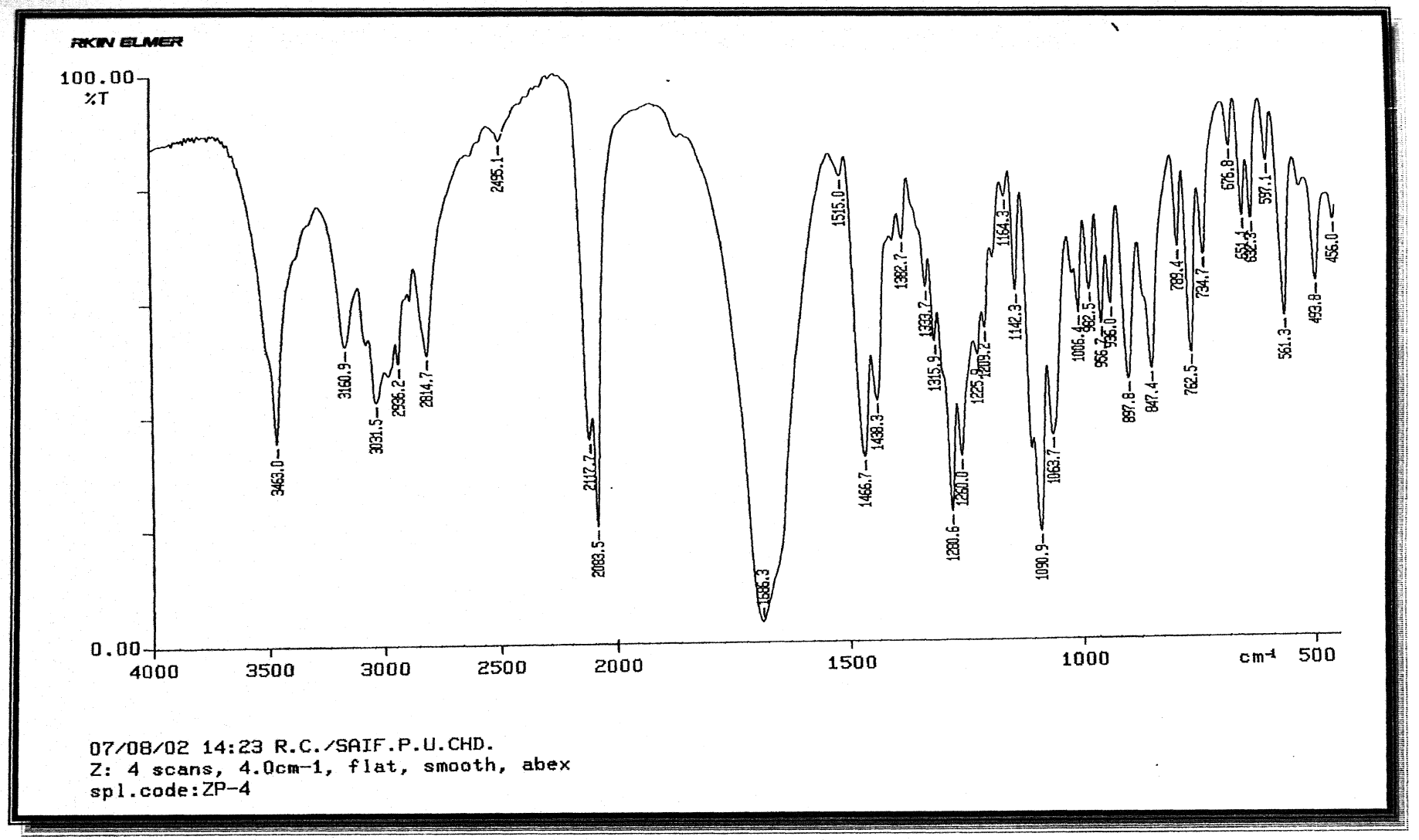


Figure-26: FTIR of the Zidovodine + Carbopol

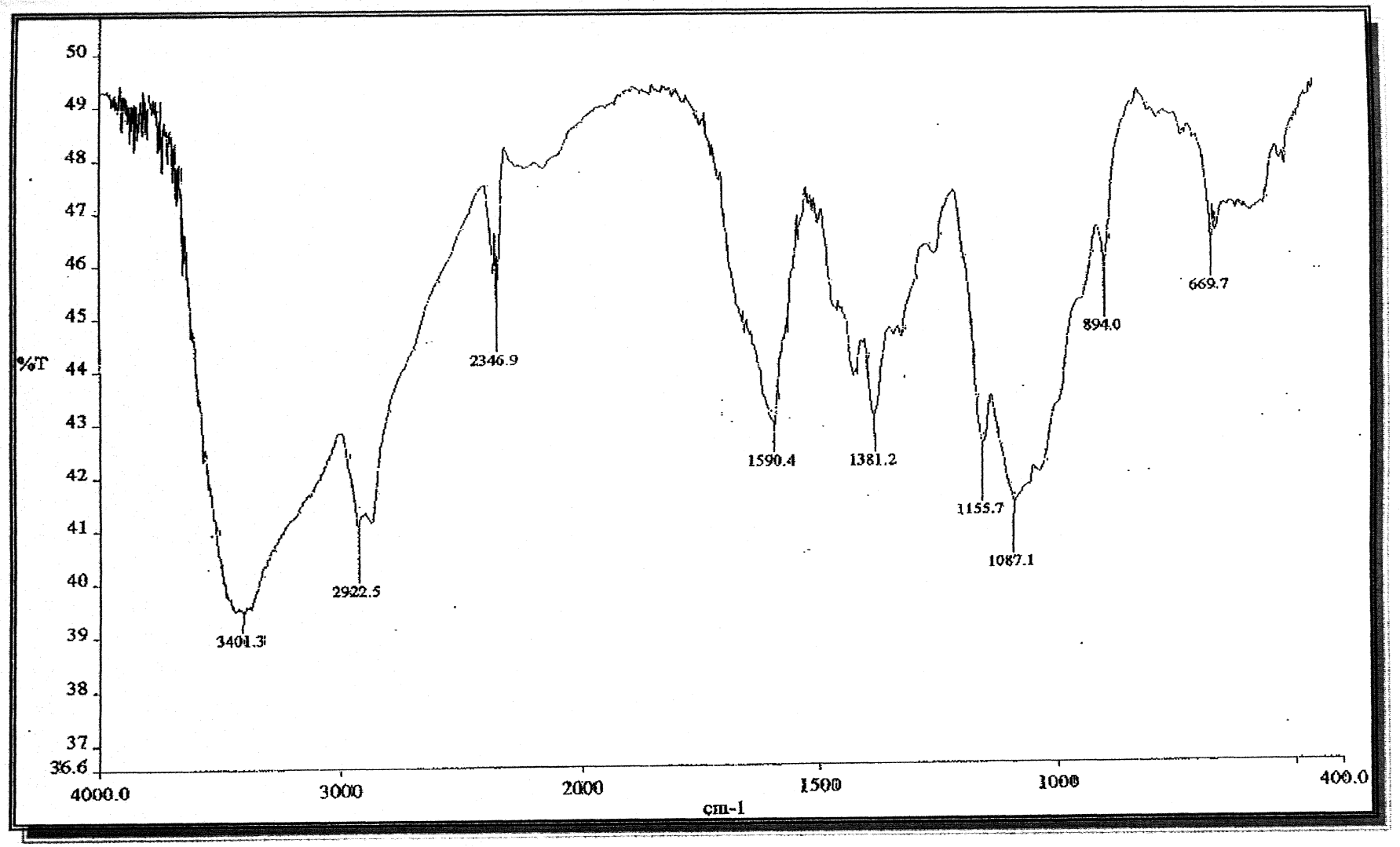


Figure-27: FTIR of the Chitosan

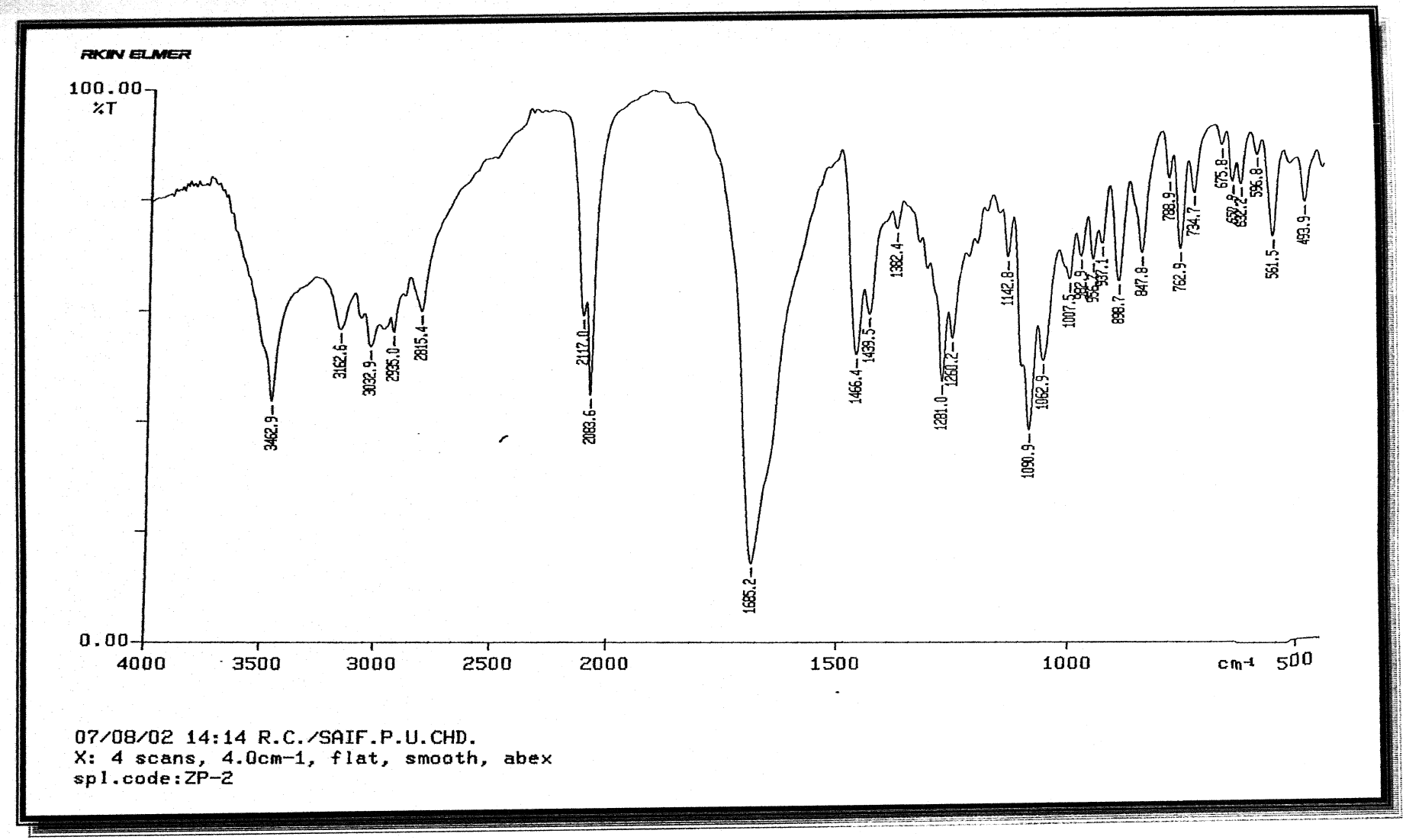


Figure-28: FTIR of the Zidovodine + Chitosan

Table –3: Interpretation of FTIR spectra of cholesterol (B)

S.No.	Peak cm^{-1}	Groups
1.	3368.7	OH stretching vibration
2.	2936.9	CH_3 as stretching vibration
3.	2867.7	CH_3 sy stretching vibration
4.	1462.6	C =C stretching vibration
5.	791	1-2 substitute benzene stretching vibration

Table –4: Interpretation of FTIR spectra of Phosphotidylcholine

S.No.	Peak cm^{-1}	Groups
1.	292.7	(CH3)3N+ Stretching vibration
2.	1081.8	C-H Stretching vibration
3.	1595.8	C=O Stretching vibration
4.	673.6	P=O Stretching vibration
5.	525.6	P-O-C aliphatic Stretching vibration

Table –5: Interpretation of FTIR spectra of Zidovudine

S.No.	Peak cm-1	Groups
1.	3245.4	OH Stretching vibration
2.	1606.1	C=O
3.	943.8	C=C Benzene ring
4.	3460.6	NH
5.	1404.1	C-N

Table –6: Interpretation of FTIR spectra of Carbopol

S. No.	Peak cm^{-1}	Groups
1.	1713.5	C=O Stretching vibration
2.	3117.7	C-H Stretching vibration
3.	2959	CH2 Stretching vibration
4.	802.5	CH Stretching vibration

Table–7: Interpretation of FTIR spectra of HPMC

S.No.	Peak cm^{-1}	Groups
1.	3664	OH
2.	2933	CH2
3.	1061	C-H bonding

Table –8: Interpretation of FTIR spectra of Chitosan

S.No.	Peak cm^{-1}	Groups
1.	3401.3	0Hstretching (NH overlapps 0H)
2.	2922.5	CH stretching
3.	1381.2	C-0-C (cyclic stretching)

Table–9: Interpretation of FTIR spectra of Polaxamar

S.No.	Peak cm^{-1}	Groups
1.	2921.8	CH3
2.	2852.5	C-H Stretching vibration
3.	1377.9	CH_3 bending
4.	1217.5	C-C-O Stretching vibration

6.1.3 Preparation of liposomes:

Liposomes with drug were prepared using the standard preparation methods. The hydrophilic drug entrapment efficiency with in the liposomes has been shown to be directly proportional to the volume of the trapped water.

The liposomes were loaded with zidovudine a water-soluble drug using the following methods.

A. Reverse phase evaporation method (RE):

i. The zidovudine was dissolved in 5 ml of water.

ii. The organic solvent (Chloroform), lipids and cholesterol were added to the water (water: organic solvent= 1:3 volume ratio)

iii. The mixture solution was sonicated for 5 minutes to form a fine emulsion.

iv. The organic solvent was removed with rotary evaporator under reduced pressure at 40±1°C to form a gel taking care not to evaporate water.

v. 20 ml of water was added to the gel and the rotary evaporation at 40± 1°C was continued to obtain liposomes[121].

B. Freeze thawing method (FTM)

i. Empty liposomes were prepared by pouring 5 ml of water into the organic solvent (chloroform) containing lipids and cholesterol. The mixture solution was sonicated for 5 minutes to form a fine emulsion.

ii. An aqueous solution of zidovudine was added to the liposome suspension at a volume ratio 1:1

iii. The solution was frozen at -20 ± 1°C for 60minutes, so that crystal phase was formed and the lipid membrane was broken.

iv. The frozen solution was thawed slowly in a 35±1°C water bath for 30 minutes.

v. The above steps 3 & 4 were repeated.

vi. The solution was sonicated for 5 minutes to obtain the liposomes[122].

C. Thin film hydration method:

i. Accurately weighed quantity of phosphotidylcholine and cholesterol in the ratio shown in the table were dissolved in chloroform and methanol mixture in 250 ml round bottom flask.

ii. A constant amount of BHT equivalent to 2% of total lipid volume was added as an antioxidant to the organic phase in the flask in some preparations.

iii. The chloroform and methanol was evaporated at 45°C under reduced pressure at 150 rpm using a rotary evaporator

iv. After the chloroform and methanol was completely evaporated.

v. The flask was kept over night under vacuum pressure to remove the remaining solvent

vi. The thin film was hydrated using saline containing drug (Zidovudine) for 2 hours until vesiculation was complete. In this method some parameters were changed to achieve maximum drug entrapment efficiency of drug in vesicles.

Table-10: Composition, Hydration time, Mean particle size, Entrapment efficiency.

S. No	PC: CH (mg)	Drug (mg)	Hydration time	Rotation time of the flask	EE	Vacuum Pressure
1.	200:200	10	02	50	35±0.8	-
2.	200:100	10	04	100	42±1.2	-
3.	200:80	10	06	150	53±1.0	2 hrs.
4.	200:80	10	08	150	59±1.6	8 hrs.
5.	200:60	10	08	150	51±1.8	8 hrs.

PC- Phosphotidyl Choline, CH-Cholesterol, EE-Entrapment Efficiency, (n=3)

The data of the table-10 showed that the thick film of lipids which gave liposomes with less entrapment of zidovudine. The thin film showed better entrapment than thick film i.e. 35±0.8% compared to the entrapment 59±1.6% obtained in liposomes prepared by thin film. The effects of the formulation variables, the lipid phase composition PC/CH and hydration condition on drug entrapment efficiency and mean particle size of liposome vesicles were studied. Decreasing amount of cholesterol in the lipid phase and increasing the drug concentration, the entrapment efficiency of zidovudine into liposomes increased[123]. The drug substance has a slight affinity for the lipid phase. The encapsulation of the zidovudine in the liposomes is related to the overall volume of aqueous phase encapsulated during liposomes formulations. Triton-X 100 is used to separate drug from the liposomes.

6.1.4 Differential Scanning Colorimetry:

The DSC studies were conducted to obtain evidences of the drug entrapment by the liposomes. The transition temperature was increased, so it confirms the drug entrapment. The phase transition temperature can give good clues about liposomal stability, permeability and whether a drug is entrapped in the bilayered or in the aqueous compartment.

DSC of Plain drug zidovudine (Single peak)

Pretransition temperature (Onset)	:	94.84°C
Main transition temperature (Peak)	:	107.50°C
Enthalpy Change(Endset)	:	117.92°C

DSC of Empty liposomes

Pretransition temperature (Onset)	:	115.42 °C
Main transition temperature (Peak)	:	1116.14°C
Enthalpy Change(Endset)	:	118.64°C
Pretransition temperature (Onset)	:	97.74°C
Main transition temperature (Peak)	:	104.79°C
Enthalpy Change (Endset)	:	112.70°C

DSC of liposomes containing drug zidovudine

Pretransition temperature (Onset)	:	98.36°C
Main transition temperature (Peak)	:	101.72°C
Enthalpy Change (Endset)	:	106.13°C
Pretransition temperature (Onset)	:	106.72°C
Main transition temperature (Peak)	:	106.36°C
Enthalpy Change (Endset)	:	108.91°C
Pretransition temperature (Onset)	:	108.87°C
Main transition temperature (Peak)	:	107.95°C
Enthalpy Change (Endset)	:	110.60°C

Differential scanning colorimetry (DSC) has been widely used to obtain values of phase transition temperature, the temperature at which the maximum peak occurs. The liposomes composed of Phosphotidyl choline and cholesterol had peaks at 104.79°C and 116°C.The liposomes changed the enthalpy of the drug. Some times the thermal behavior of the biomembrane phase transition is affected by presence of foreign molecules.

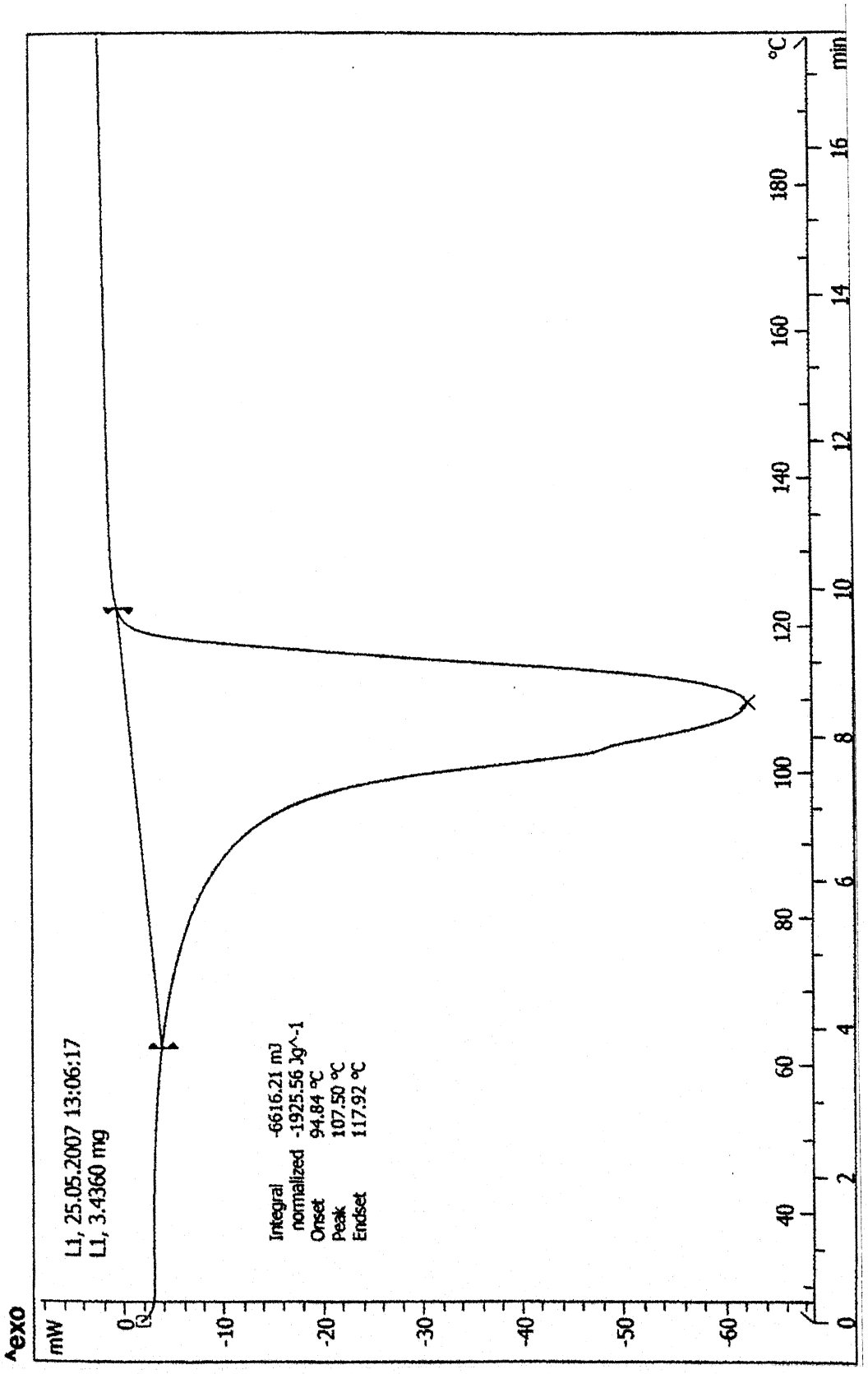


Figure-29: DSC of Zidovudine

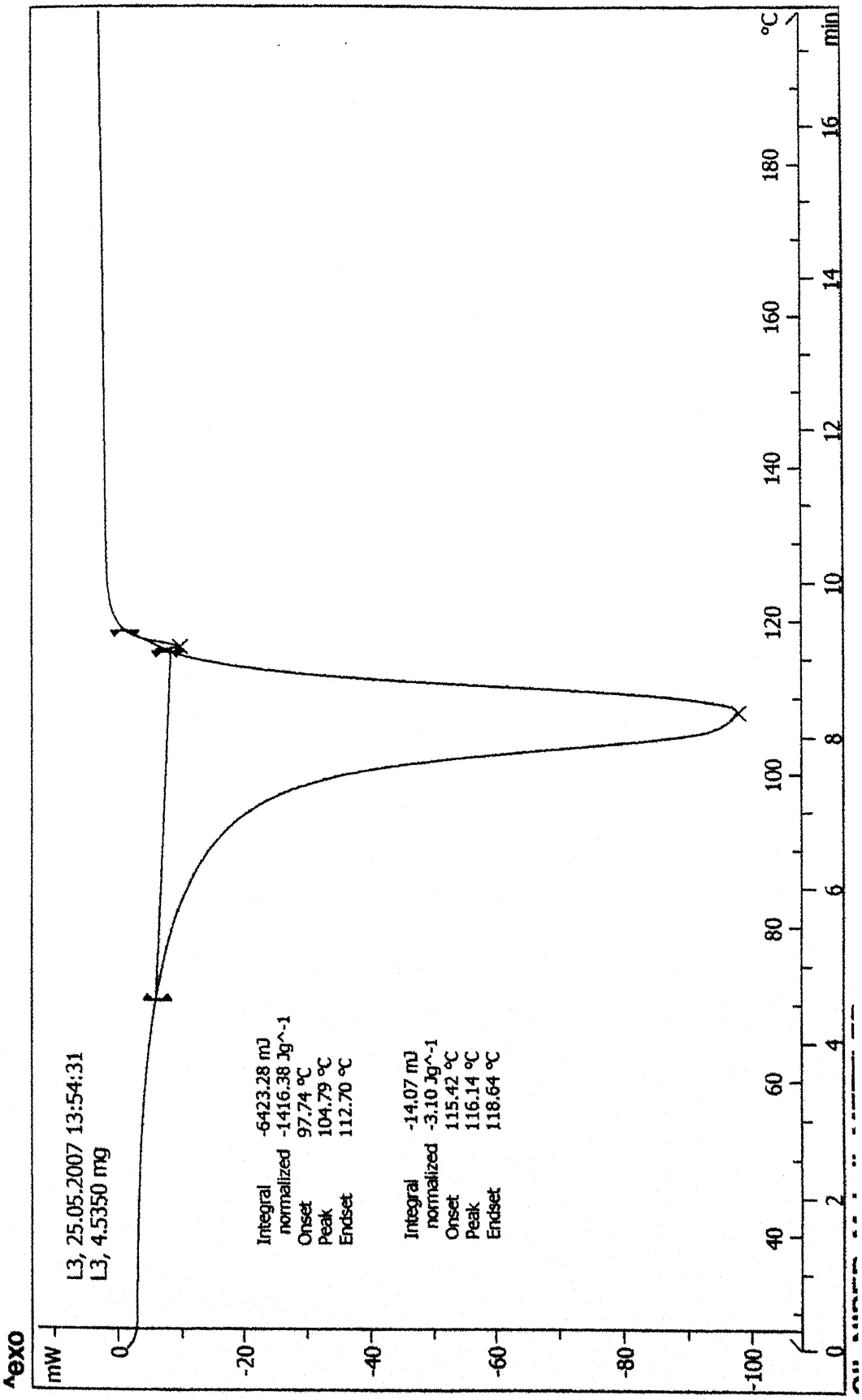


Figure-30: DSC of Empty liposomes

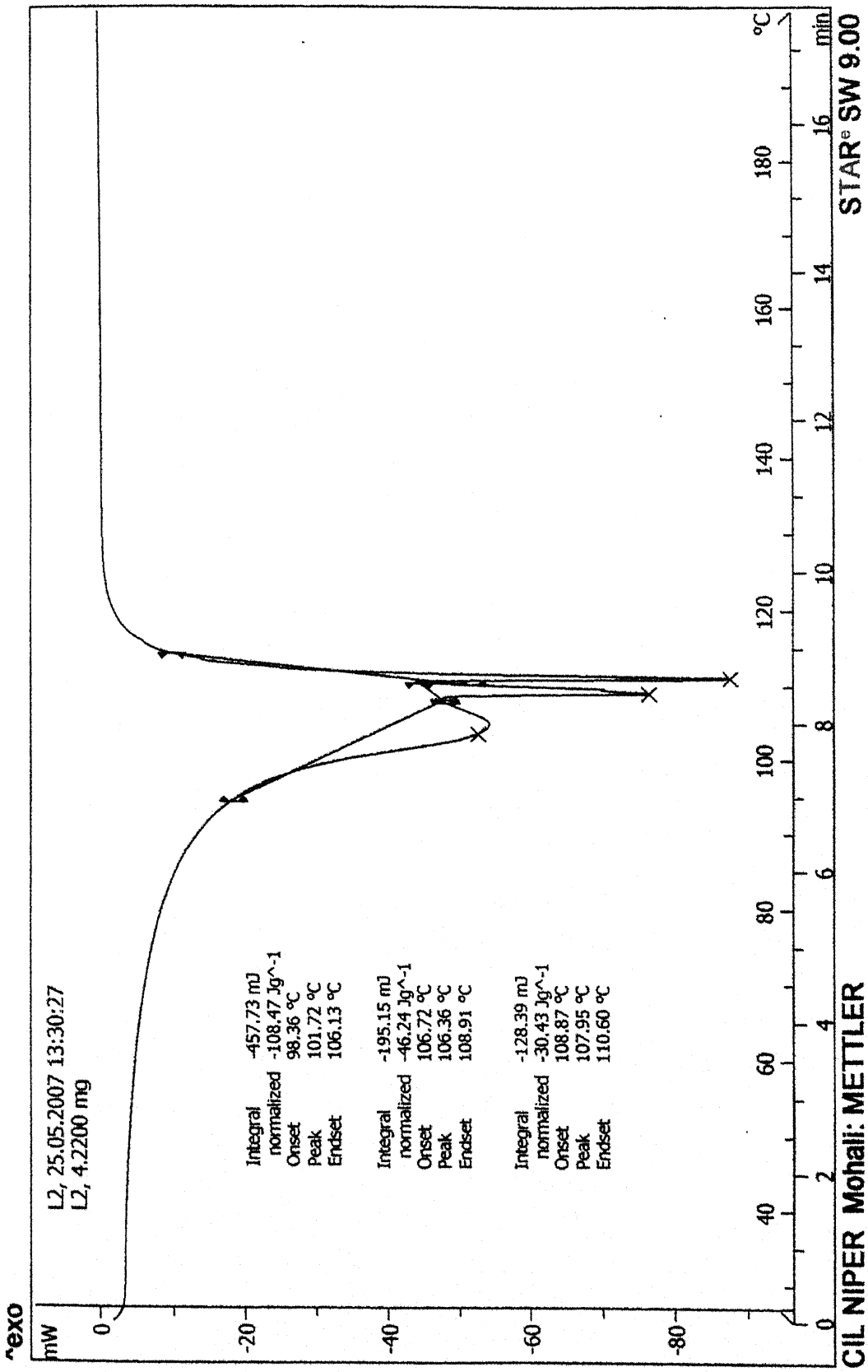


Figure-31: DSC of Zidovudine liposomes

6.1.5 Drug release from the conventional liposomes:

The drug release from the conventional liposome formulations is present in the table-11and fig-32. It was found that 98.64% of encapsulated drug was released during a period of 8 hours, in 37°C temperature. The drug release from aqueous solution also conducted by using dialysis membrane. It was found that 99.62 percent of drug released from the aqueous solution containing Zidovudine.

Table-11: Percentage drug release from aqueous solution.

S. No	Time in hours	% Drug release from aqueous solution
1.	0.5	57.86
2.	1	74.5
3.	1.5	82.6
4.	2	88.2
5.	2.5	96.5
6.	3	99.62
7.	3.5	99.81

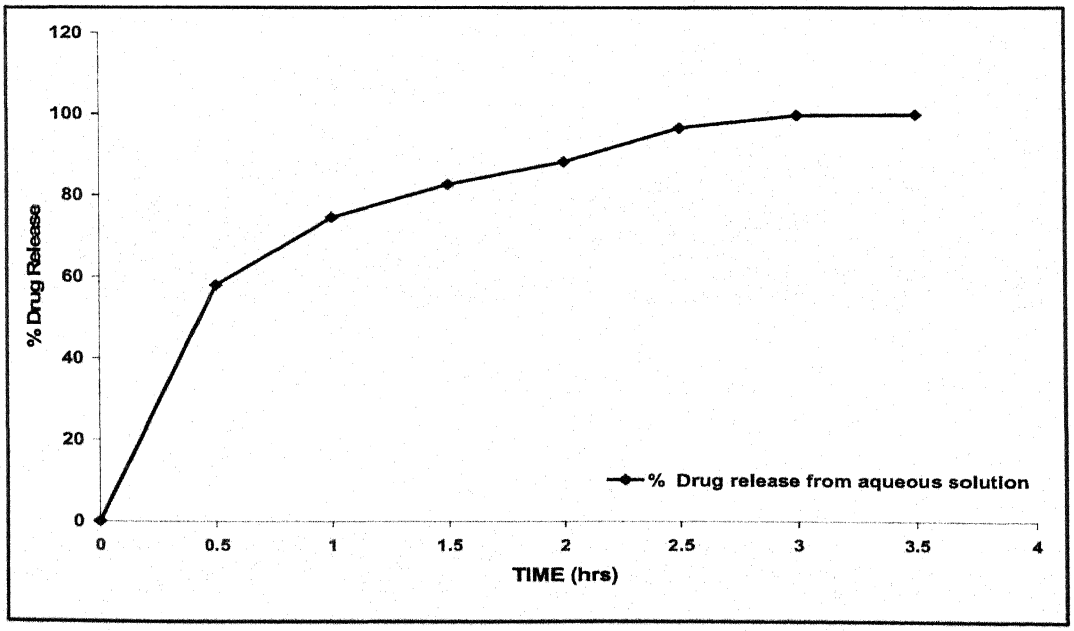


Figure-32: Percentage drug release from aqueous solution.

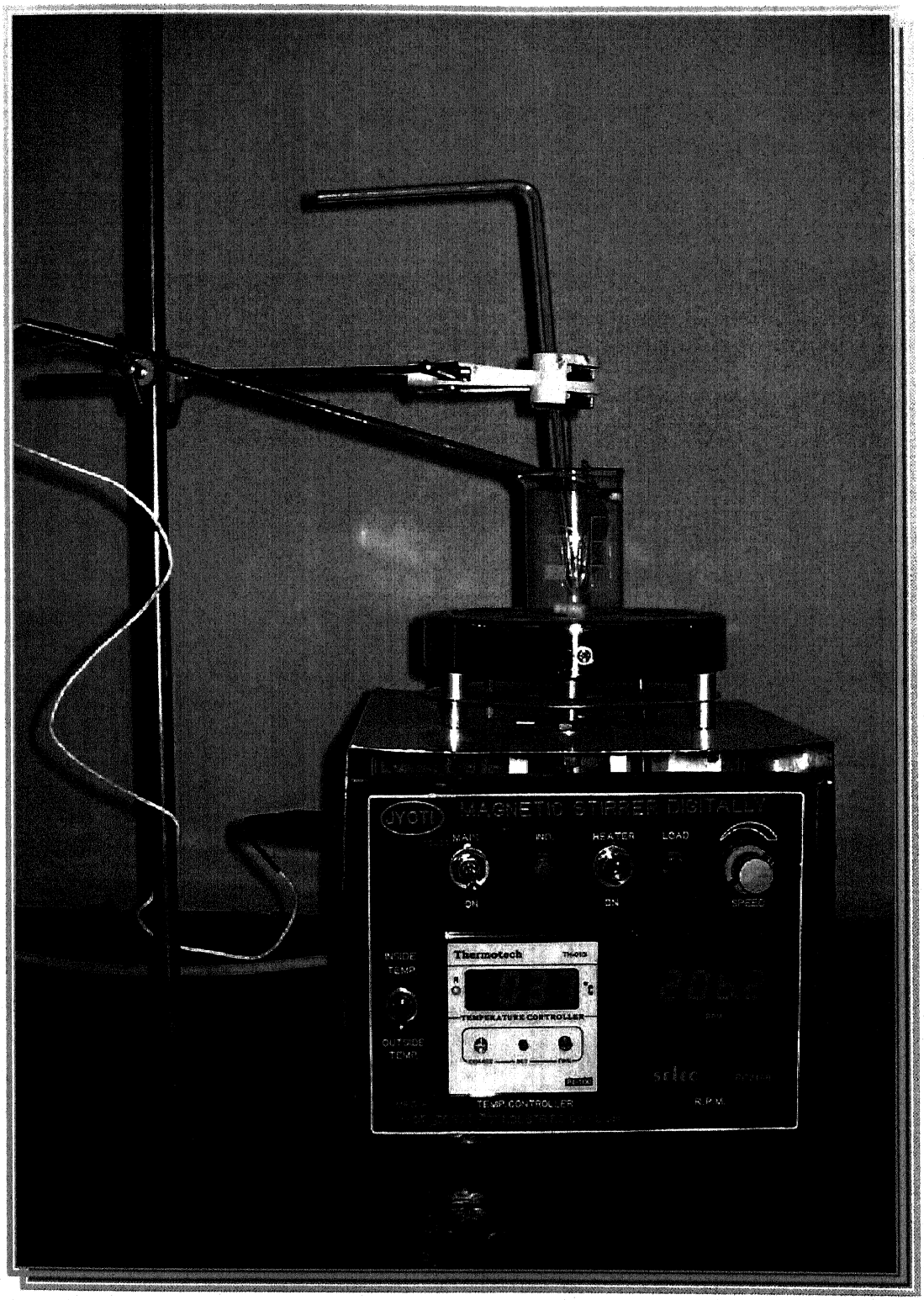


Figure-33: Picture showing drug release from Dialysis Membrane (Bag)

Table-12: Percentage drug release model from Aqueous Solution.

Model	Percentage release model from Aqueous solution	
	Drug release from Aqueous Solution	
Zero Order	R	0.7116
	K	19.7299
	SSQ	1141
1st Order	R	0.8479
	K	-0.2750
	SSQ	599
Matrix	R	0.9753
	K	32.3512
	SSQ	113
Peppas	R	0.9971
	K	37.0614
	SSQ	04
Hix Crow	R	0.8080
	K	-0.0816
	SSQ	744
		Best fit Model-PEPPAS

Table-13: Percentage drug release from Aqueous Solution.

RESULTS			Zero	1st order	Matrix	Peppas	Hix.Crow.
Sr. No.	Time	Avg. %R	1141	599	113	4	744
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	0.5	28.931	363.496	258.750	36.660	0.231	295.033
3.	1	37.839	327.924	190.408	30.111	0.604	233.776
4.	1.5	42.628	169.864	77.994	9.036	0.040	104.095
5.	2	46.254	46.162	15.632	0.253	0.201	23.325
6.	2.5	51.291	3.865	2.493	0.019	0.960	2.904
7.	3	53.807	28.968	5.587	4.957	0.117	9.897
8.	3.5	54.898	200.410	47.650	31.647	1.929	75.115

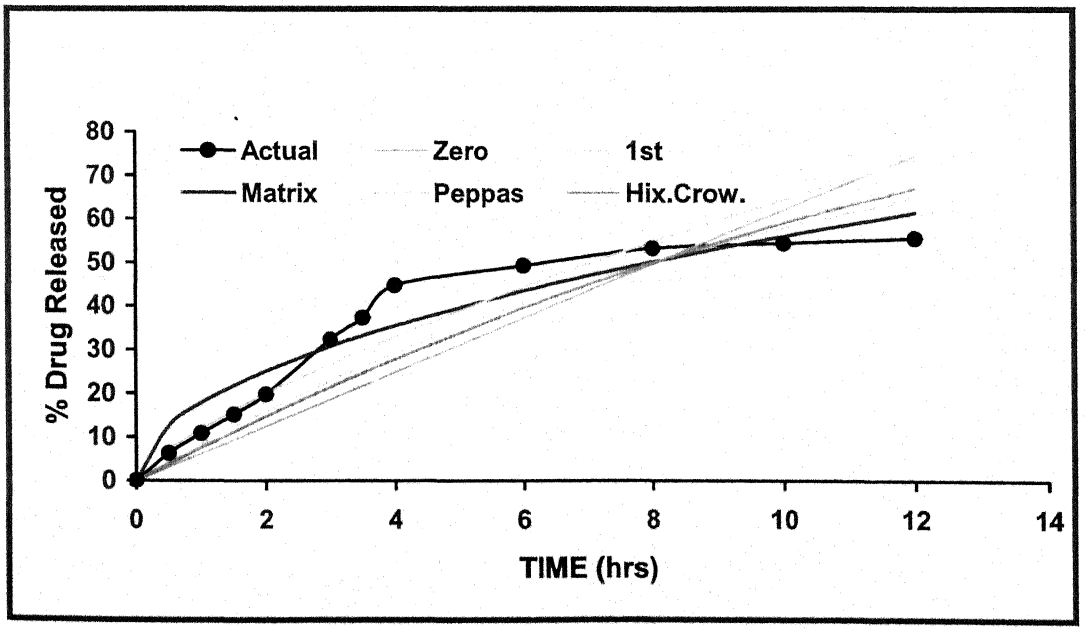


Figure-34: Percentage drug release from Aqueous Solution.

Table-14: Drug release from conventional liposomes

S. No	Time in hours	% Drug release from conventional liposomes	S. No.	Time in hours	% Drug release from conventional liposomes
1.	0.5	12.5	7	3.5	71.25
2.	1	21.45	8	4	84.6
3.	1.5	29.56	9	6	92.01
4.	2	38.2	10	8	98.23
5.	2.5	50.74	11	10	98.56
6.	3	62.48	12	12	98.99

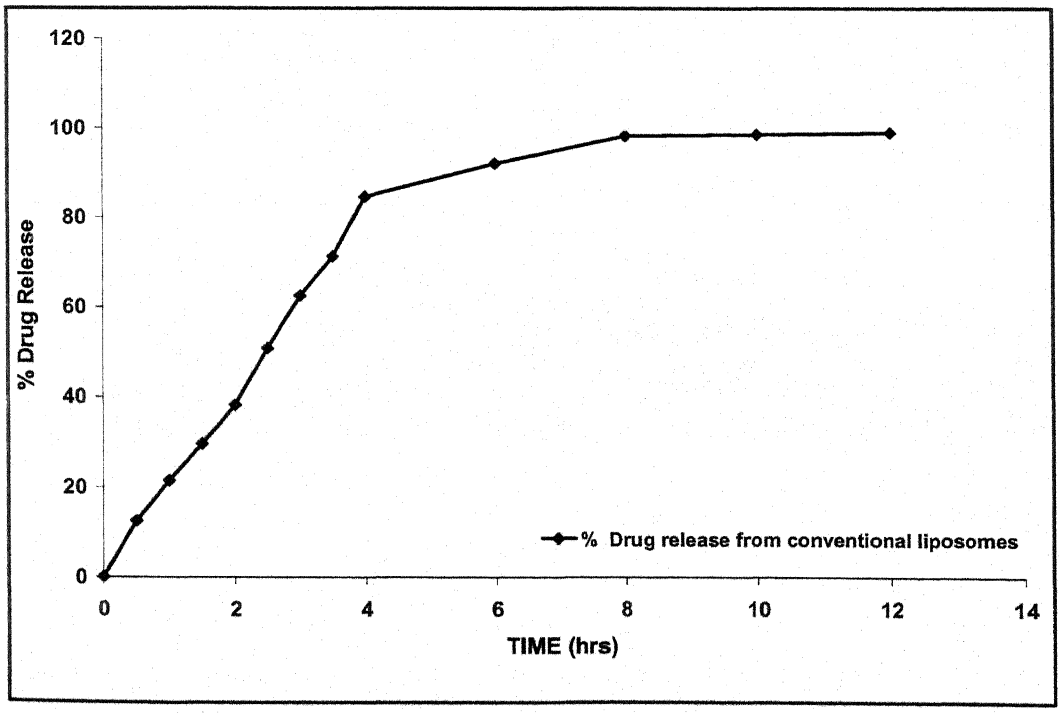


Figure-35: Percentage drug release from conventional liposomes.

Table-15: Percentage drug release from conventional liposomes.

Model	Drug release from conventional Liposomes	
	Conventional liposomes	
Zero Order	R	0.5160
	k	3.5327
	SSQ	5787
1st Order	R	0.7450
	k	-0.0526
	SSQ	3240
Matrix	R	0.9273
	k	14.9438
	SSQ	1105
Pappas	R	0.9393
	k	13.8545
	SSQ	1564
Hix Crow	R	0.6848
	k	-0.0152
	SSQ	3939
		Best fit-PEPPAS

Table-16 : Percentage drug release from conventional liposomes.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	5787	3240	1105	1564	3939
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	0.5	6.250	20.106	13.366	18.632	10.292	15.855
3.	1	10.972	55.337	34.224	15.778	8.311	41.851
4.	1.5	15.122	96.499	56.843	10.112	4.831	70.904
5.	2	19.733	160.475	95.113	1.961	0.314	118.064
6.	2.5	26.397	308.551	198.282	7.668	11.906	237.100
7.	3	32.768	491.488	330.391	47.393	54.721	386.910
8.	3.5	37.778	645.845	439.788	96.446	103.226	511.451
9.	4	45.102	959.203	683.124	231.474	236.438	778.805
10.	6	49.712	813.141	513.424	171.800	157.509	610.747
11.	8	53.751	649.703	377.033	131.872	104.251	460.313
12.	10	54.850	381.135	194.944	57.660	31.543	247.927
13.	12	56.055	186.672	85.895	18.392	2.632	112.691

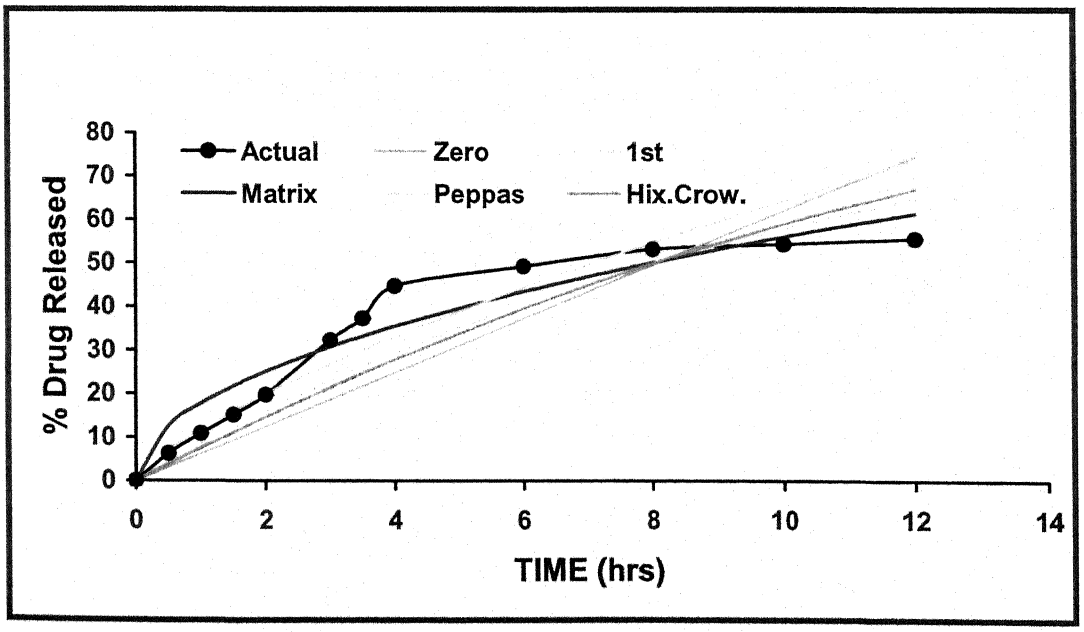


Figure-36: Percentage drug release from conventional liposomes.

6.1.6 Estimation of Entrapped Drug in Liposomes:

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15 000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained. The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer).

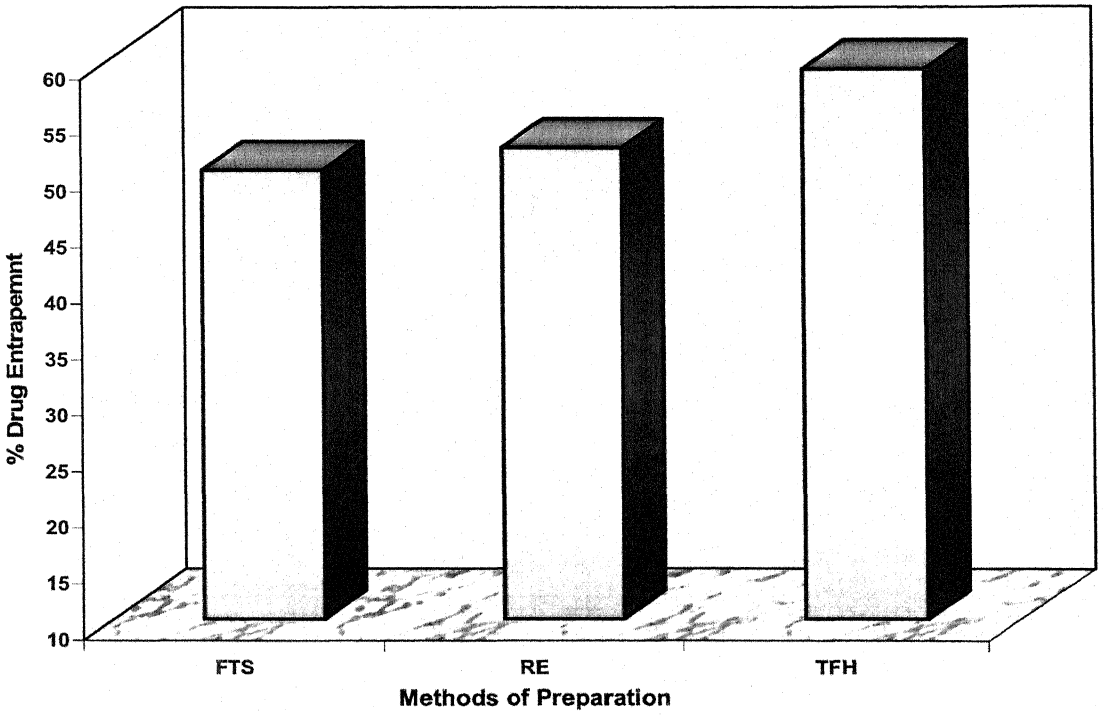


Figure-37: Percentage drug entrapment

Encapsulation efficiency was calculated according to equation.

Encapsulation efficiency = (A1 – A2)* 1OO/A1

Where,

A1= Amount of zidovudine added initially

A2= Amount of zidovudine determined in the filtrate by spectrophometry.

(A1-A2)= represents the amount of zidovudine trapped in the liposome formulation.

This should equal the total amount added initially, minus the amount determined in the filtrate.

The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant. There are two methods to find out the entrapment efficiency, the first one is by using the methanol and second one is by using detergent like TritonX-100.

The amount of drug exactly present within the liposomes was also analyzed by dissolving the liposomes in methanol to countercheck the percentage drug entrapment and to arrive at a mass balance. The analysis of drug in liposomes was carried out using the empty liposomes dissolved in methanol as blank in order to nullify the interference of the excipients.

6.1.7 Encapsulation efficiency:

The pellets obtained from centrifugation were resuspended in 0.9% w/v saline solution. The influence of detergent on liposomes solubilisation was determined by dissolving 300µl of liposome suspension with 1%, 2%, 3%, 4%, 5%, and 6% with different concentrations to find out the exact concentration of Triton X-100 detergent. The detergent solution was then gently heated for 5 minutes. The clear solution was quantitatively analyzed for zidovudine by spectrophotometery at 267nm. Triton-X 100 (5%) is used to separate drug from the liposomes. Below and obove5% showed less drug concentration. So, a 5% triton-X 100 concentrations is suitable to find out the drug entrapment in the liposomes.

Table-17: Percentage of liposomes solubilisation by detergent Triton X-100

S. No.	% of Triton X-100	% of liposomes solubilisation
1.	1%	26.45
2.	2%	48.56
3.	3%	67.84
4.	4%	89.3
5.	5%	100
6.	6%	92.02

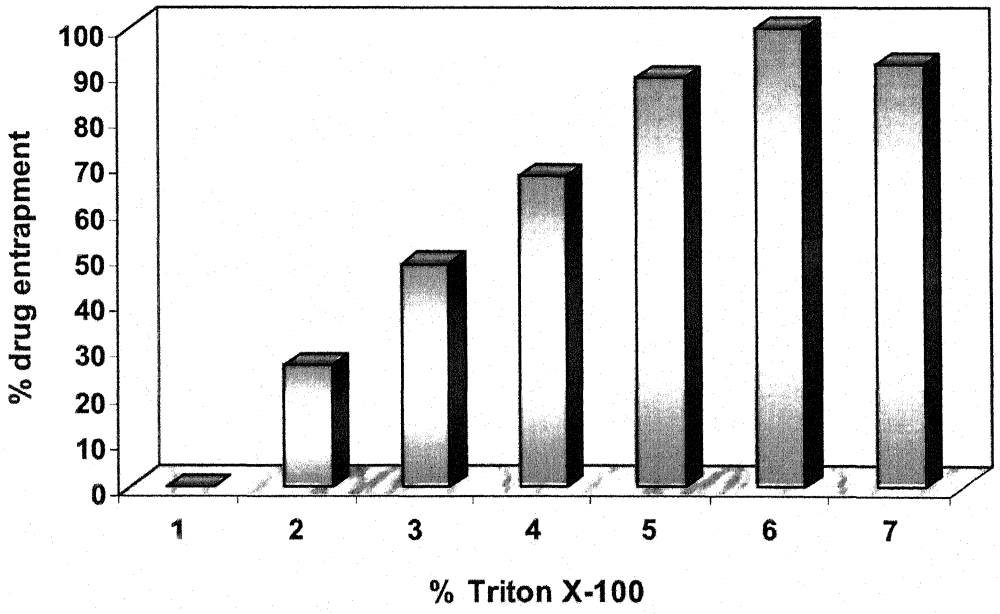


Figure-38: Percentage of liposomes solubilisation by detergent Triton X-100.

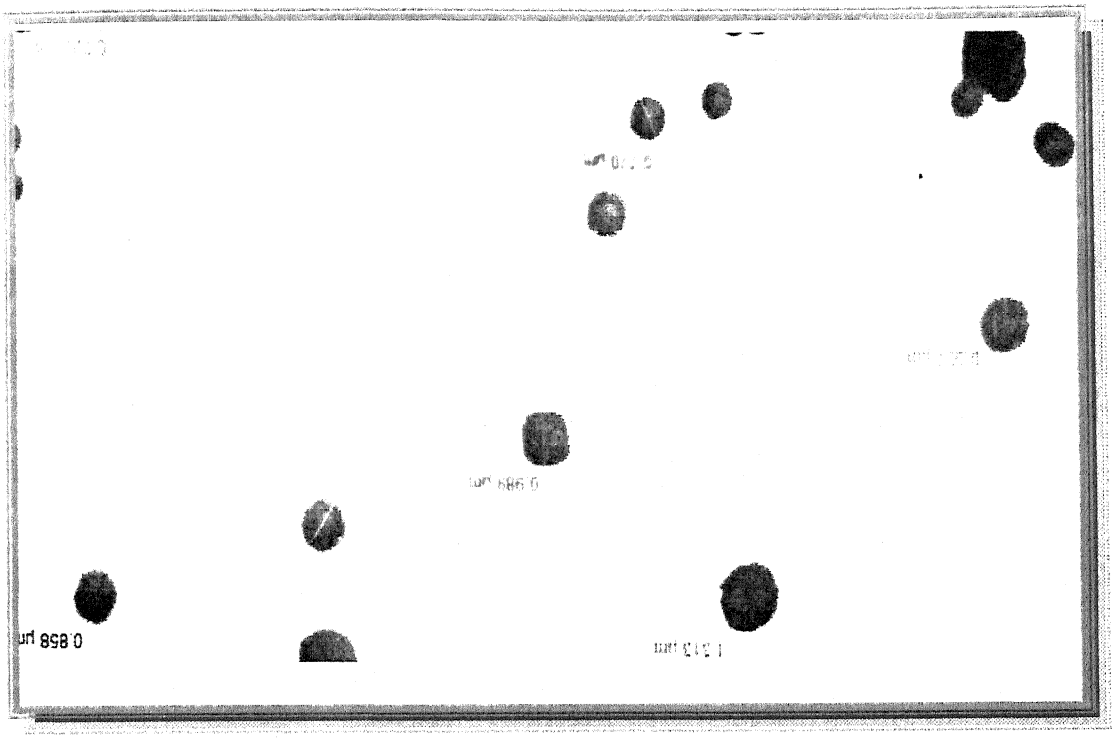


Figure-39: TEM of Liposomes Loaded with Zidovudine.

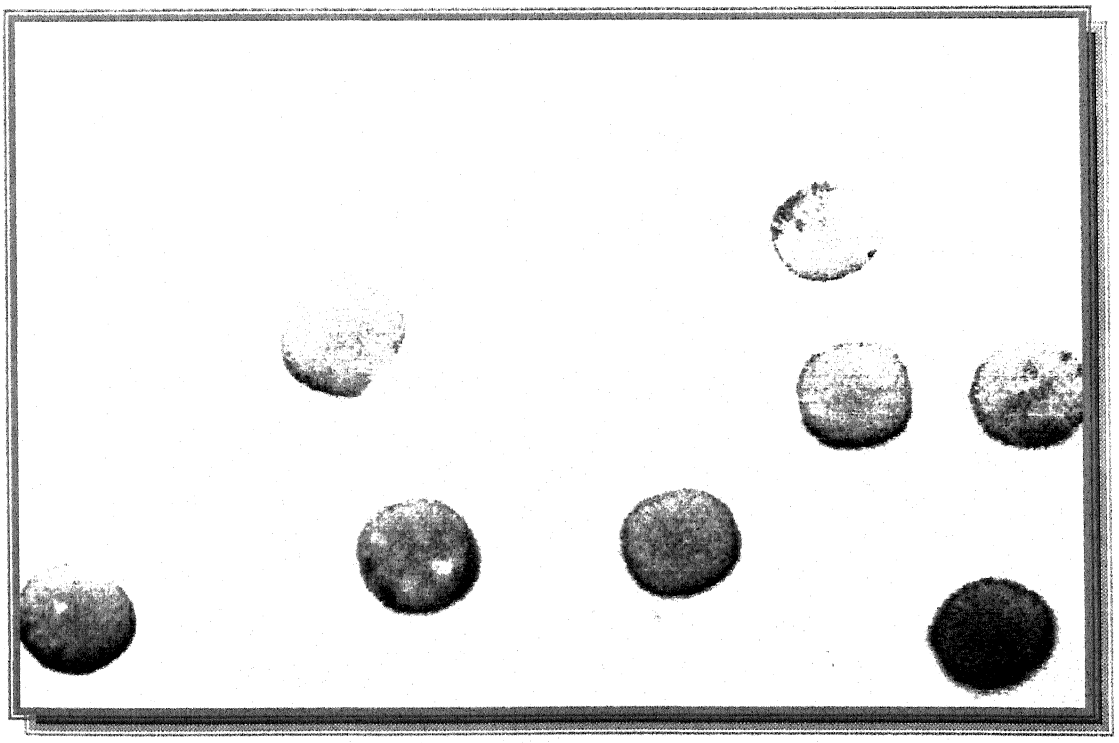


Figure-40: Enlarged TEM of Empty Liposomes (without drug).

Figure-41: TEM of Liposomes loaded with Zidovudine.

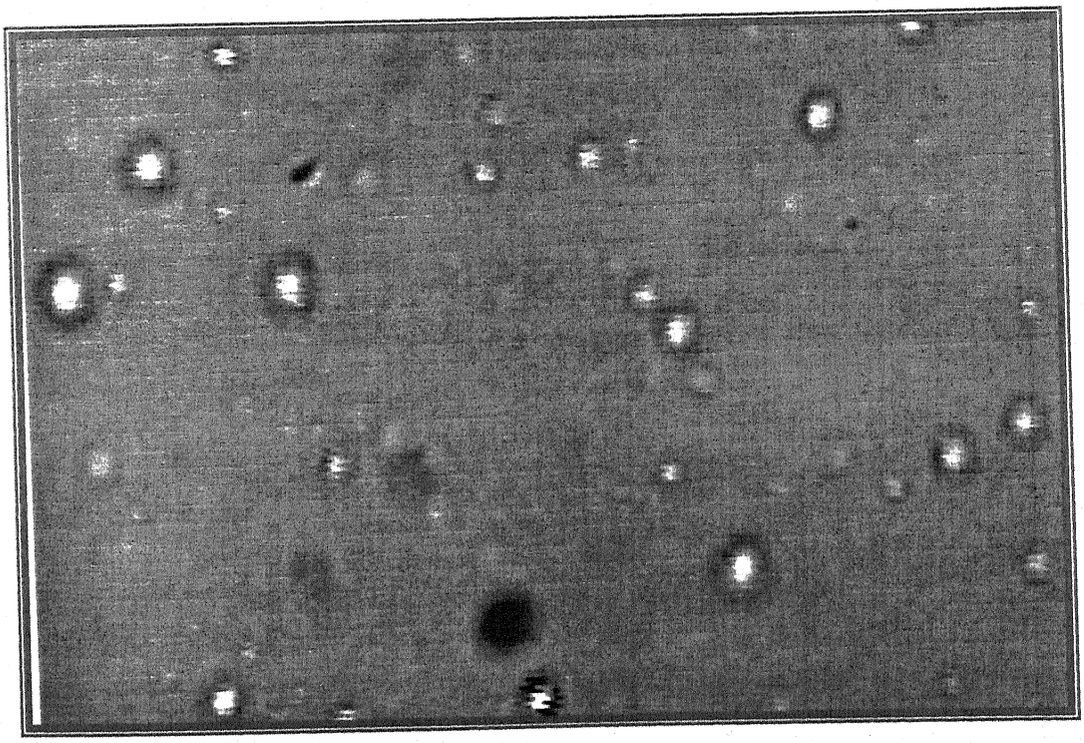


Figure-42: Optical microscopic photograph of gel containing liposomes.

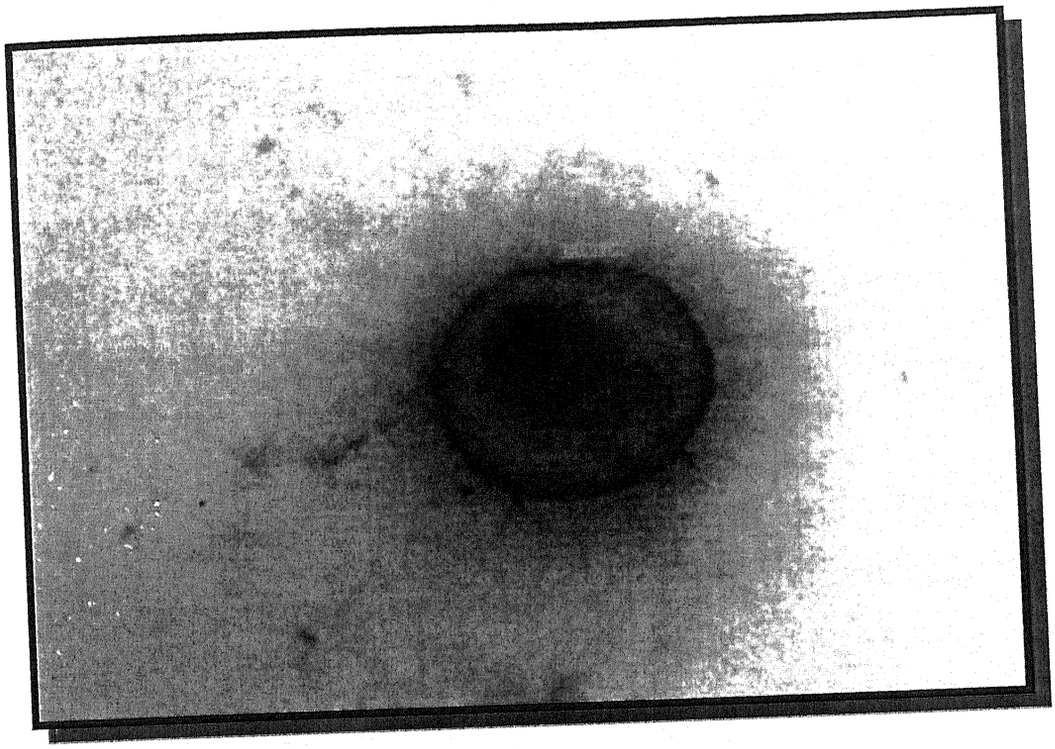


Figure-43: TEM of Single Liposome loaded with Zidovudine.

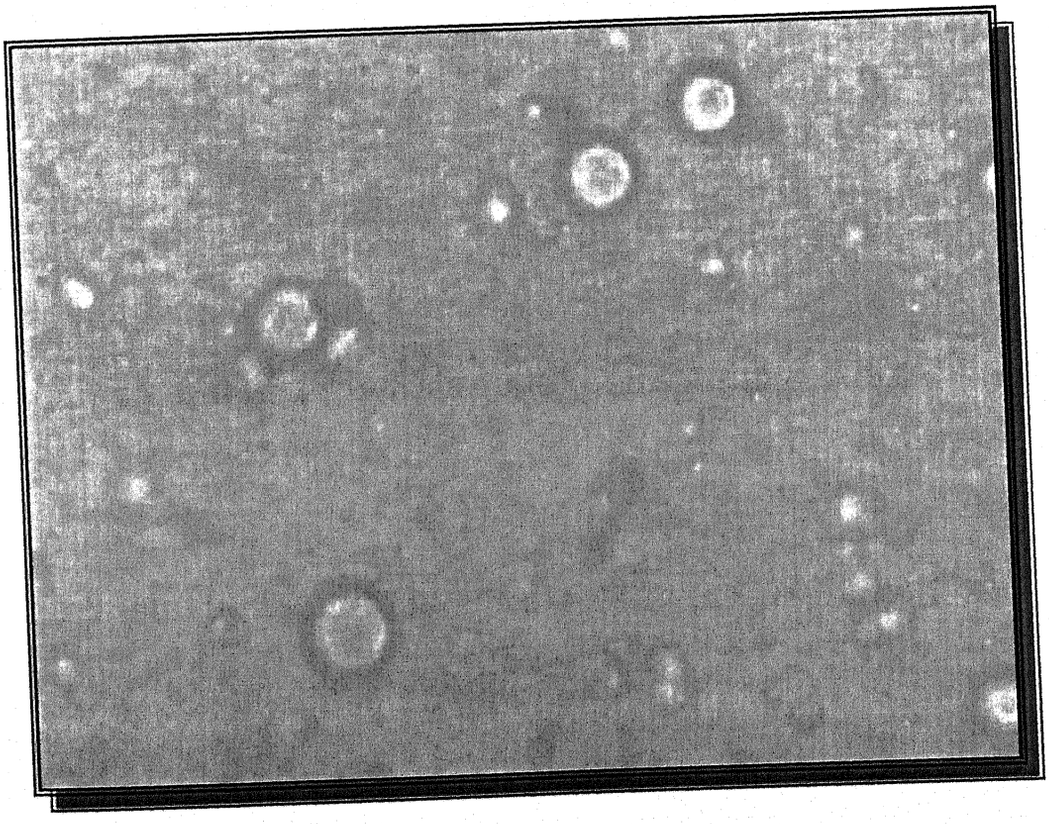


Figure-44: Optical microscopic photography of the liposomes.

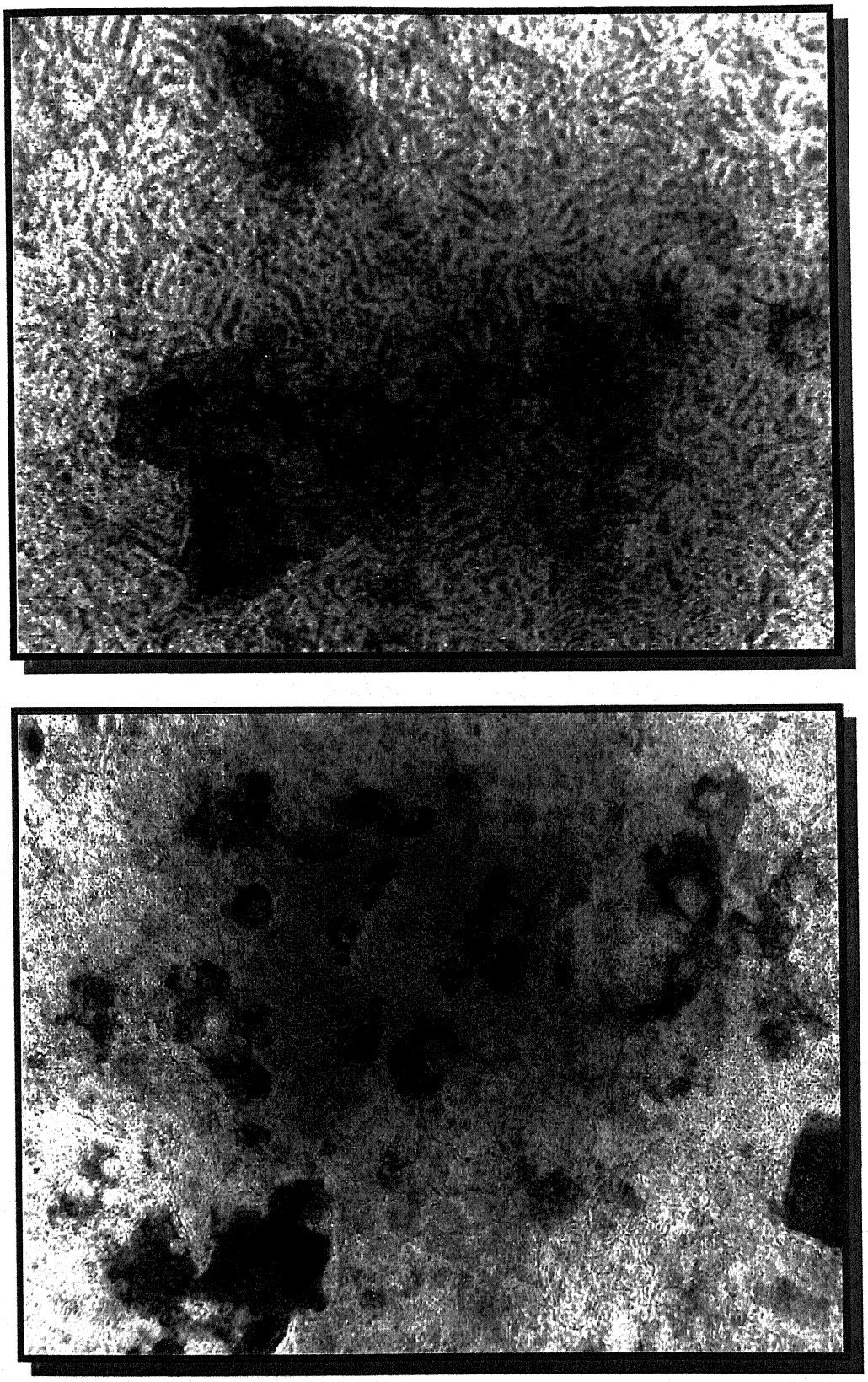


Figure-45: TEM of Dried Liposomes.

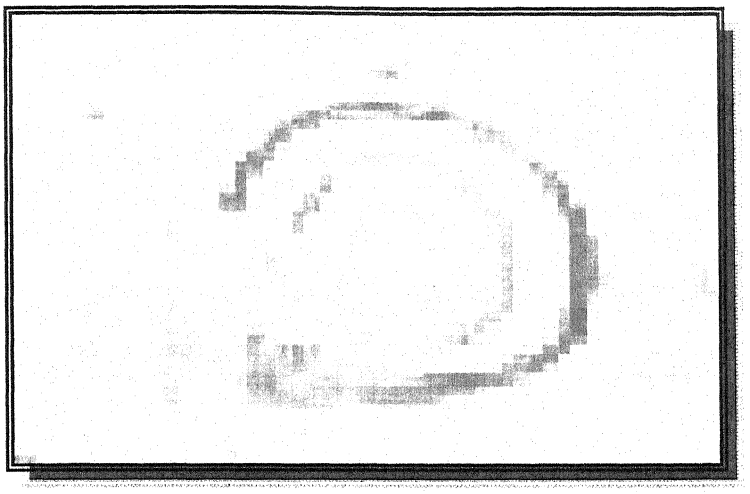


Figure-46: Shows mMultilammellar vesicles

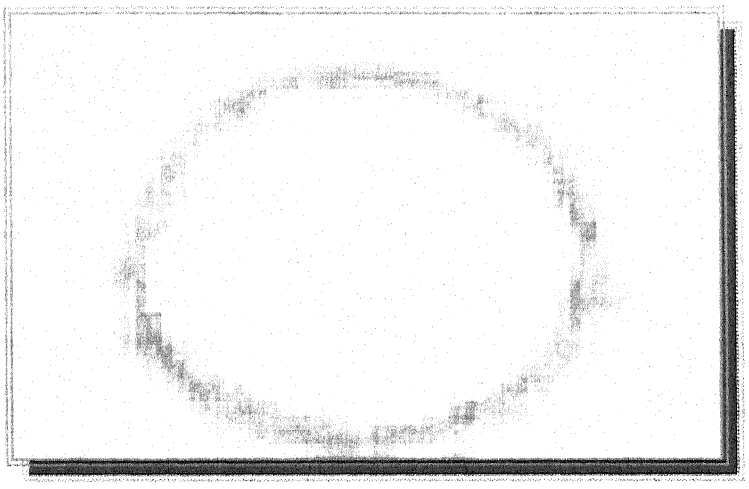


Figure-47: Liposome vesicle before drug release

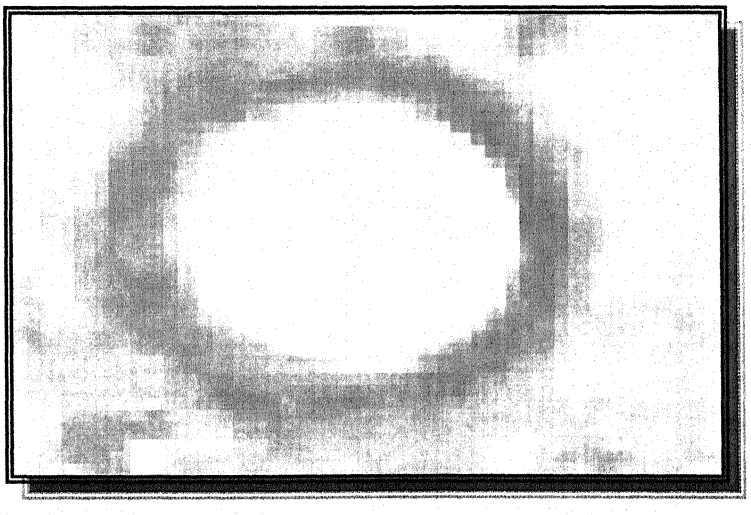


Figure-48: Liposome vesicle after drug release

6.1.8 Validation of the method of the spectrophotometric estimation of Zidovudine:

The method was validated by recovery studies. By adding known quantity of standard to the sample preparation estimating the quantity of drug recovered in the preparation. The following solutions were prepared and absorbance measured at 267 nm.

A. Standard solution :

10mg of zidovudine was accurately weighed and transferred in 100 ml standard flask. The drug was dissolved in water and volume was made up with same, this solution labeled as stock solution (standard). From the above stock solution 5 ml was pipette out and transferred in to 100 ml standard flask and volume was made up with water. The final concentration of the solution was 5 ug/ml.

B. Sample solution :

10mg equivalent zidovudine containing liposomes was accurately weighed and transferred into a 100 ml flask. Saline solution was added and made to 100mlflask. Saline solution was added and made 100 ml, resulting solution was filtered. From the filtrate, 10ml was pipetted out and transferred to 100ml standard flask and volume was made with saline solution. The final concentration of the solution was 10 mcg/ml.

C. Sample solution for recovery studies:

Test solution and standard solution was pipette out and transferred into the 100 ml flask. The final concentration of the solution would be around 20 mcg/ml.

Since the absorbance values are additive, the absorbance values of test and standard samples should be equal or (should not differ) to the absorbance of sample prepared for the recovery studies as both have same quantity of zidovudine. On the basis it was found that percentage zidovudine recovered to be99.02%.

$$\% \text{ Recovered} = \frac{\text{Absorbance of sample prepared for recovery studies}}{\text{Absorbance of standard} + \text{Absorbance of sample}} \times 100$$

$$= \frac{0.253}{0.126 + 0.120} \times 100$$

$$= \mathbf{99.02\%}$$

Hence the assay procedure used for determining zidovudine from the liposomes is validated and can be used for content determination.

6.1.8.1 Osmotic study:

0.1 ml volume of dispersed liposomes was mixed with NaCl solutions at various concentrations to get a desired concentration gradient across the lipid layer membranes. Samples were incubated for one hour and then the turbidity of the mixture was measured at 450 nm. The experimental data were analyzed in order to verify through the osmotic behavior the reconstitution of the liposomal structure after hydration.

Under the hypotonic conditions, the plot deviated from the linear relationship since the liposomes were lysed, the solute leaked, and liposomal membrane lost its membrane barrier function. Under hypertonic conditions, the liposomes might have shrunk and absorption rate deviated from linearity. Under more hypotonic conditions the plot deviated from the linear relationship since the liposomes were lysed, the solute leaked, and the liposome membrane lost its barrier function. Under hypertonic condition, the liposomes might have shrunk and the absorption data deviated from linearity. The similarity between the linear relation ships in both hyper (shrunk) and hypotonic (membrane lost its barrier) graphs shows that the lipid aggregates.

Effect of Cholesterol on Encapsulation efficiency at different pH

The effects of Cholesterol on encapsulation capacity and the sizes of liposomes varied with preparing Conditions and the properties of encapsulated materials. Zidovudine was incorporated in to the liposomes containing Phosphotidyl choline and Cholesterol at high rates under the conditions studied. There was a trend for the entrapment efficiency of zidovudine into liposomes to increase with increasing CH content at all different pH, Saline solution and aqueous conditions tested. Effect of solution pH and cholesterol content on the efficiency of incorporation of Zidovudine into liposomes.

Table- 18: Effect of solution and cholesterol content on the efficiency of incorporation of zidovudine in Liposomes

S.No.	Ratio of CH and PC	Water	Saline	pH5.0	pH7.0	pH8.0
1.	200:00	40± 1.2	38±1.1	37±1.2	38.2±1.4	39.5± 1.1
2.	200:20	41± 1.4	38±1.2	36.8± 1.8	38.9±1.5	40± 1.4
3.	200:40	50± 1.1	47.1±1.4	43± 1.2	43±1.7	46.1±1.5
4.	200:60	51±1.8	50±1.1	51± 1.4	52±1.1	57.2±1.4
5.	200:80	59±1.6	57±1.3	54±1.6	56±1.6	58±1.3
6.	200:100	42±1.2	40±1.4	41±1.5	41±1.5	42±1.1
7.	200:200	35±0.8	33±1.6	33.7±1.2	34.2±1.2	35±1.5

n=2

The mean size of the liposomes also increased with increasing cholesterol content. The sizes of the liposomes were observed under the optical microscope after the preparation (without vortexing or Sonicated). These results are in line with the idea that the size of the liposomes increase with increasing cholesterol content.

Table-19: Effect of cholesterol content in liposomes on the size of zidovudine incorporated liposomes

S. No.	Ratio of CH and PC	Water	Saline	pH5.0	pH7.0	pH8.0
1.	200:00	2.13±0.08	2.21±0.04	3.14±0.04	2.31±0.04	2.45±0.07
2.	200:20	2.24±0.06	2.29±0.06	3.24±0.05	2.39±0.07	2.59±0.08
3.	200:40	2.48±0.10	2.34±0.08	3.37±0.06	2.43±0.06	2.65±0.06
4.	200:60	2.76±0.07	2.56±0.09	3.41±0.01	2.48±0.04	2.83±0.02
5.	200:80	2.98±0.06	2.75±0.07	3.54±0.07	2.55±0.08	2.92±0.12
6.	200:100	3.12±0.05	2.89±0.05	3.72±0.06	2.88±0.10	3.12±0.10
7	200:200	4.42±0.08	3.26±0.02	3.96±0.02	3.21±0.09	3.51±0.12

n=2

6.1.9 Liposomes stability in plasma

The stability of liposomes in human plasma was evaluated by 200µl of empty liposomes in 1 ml of plasma at 37°C and the turbidity was measured under spectrophotometer (450nm) at different time intervals like 1,2,4,6,8,10,12,24 hours.

Table-20: Stability of liposomes in plasma.

S.no	Time in hours	Absorption at 450nm
1.	1	0.76
2.	2	0.784
3.	4	0.798
4.	6	0.799
5.	8	0.812
6.	10	0.823
7.	12	0.821
8.	24	1.12

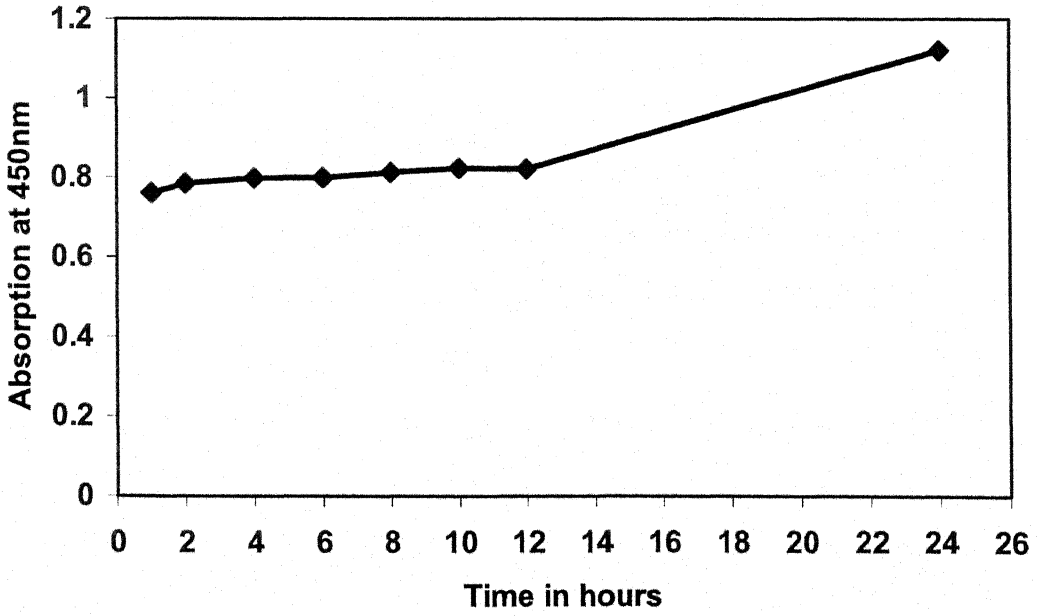


Figure-49: Shows the stability of liposomes in plasma.

The results obtained on plasma stability when the liposomes were exposed to human plasma solutions. When dispersions were exposed to plasma, large changes in absorbance indicated instabilities due to slow fusion and aggregates might occur.

6.2 DRUG RELEASE FROM LIPOSOMES:

Drug release test is a basic part of drug development and formulation development and is also employed as a quality control tool to monitor batch to batch consistently of the drug release from liposomes.

6.2.1 Dialysis bag method:

Zidovudine release from liposomes was determined by using the dialysis bag. The dialysis bag membrane is permeable to release medium and it can allow the drug such as zidovudine. Before using the dialysis bag was soaked in water for around four hours to remove the contaminants and debris in the membrane. A 4.0 ml of zidovudine liposomal suspension and empty liposomal suspension separately was taken in to beakers containing 100ml water was the drug release medium. The medium temperature was maintained at 37°C prior to use, in order to de aerate the medium, the stirring speed and temperatures were maintained at 100±10 rpm and 37±0.5°C respectively. An aliquot of 2ml sample was withdrawn at different time intervals for the determination of zidovudine concentration by U-V spectroscopy at 267 nm. At the same time, same volume of water was replaced to maintain the sink condition.

The empty liposomes was taken to found that the absence of zidovudine, no λ max at 267 nm was measured in solvent, which indicates that the liposomes constituents do not interfere with the measurement of zidovudine concentration. The above release test was to confirm the interference of phospholipids or other substances.

6.2.2 Effect of temperature on the release of drugs from liposomes:

The temperature of the release medium may alter the viscosity of the liposomal suspension. Generally the human body temperature is 37°C. However in

the clinical cases, such as fever, the variation of temperature can change the drug release when liposomes are sensitive to the temperature. The fig-50 shows that increasing temperature of the medium increases zidovudine release from the conventional liposomes. The release studies were conducted for 2 hours to find the behavior of liposomes at different temperature. Stirring speed kept constant at 100rpm. The temperature of the medium has no significant effects on zidovudine release from liposomes in the range 37-39°C. This means that drug release from the liposomes in non-temperature sensitive invitro at body temperature. But with increased temperature, the zidovudine release rate increased. This effect was because of the rising temperature increases the value of diffusion.

Table-21 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

S. No.	Time in minutes	% Drug released at 25°C	% Drug released at 37°C	% Drug released at 39°C	% Drug released at 50°C
1.	0	0	0	0	0
2.	10	2.53	6.24	7.51	18.02
3.	20	7.42	16.03	18.62	26.32
4.	30	10.54	21.54	22.67	35.45
5.	40	14.65	23.99	25.87	43.87
6.	50	16.87	24.87	28.36	47.85
7.	60	20.41	27.8	30.84	53.01
8.	90	25.28	32.69	36.44	61.22
9.	120	29.98	38.54	42.57	72.42

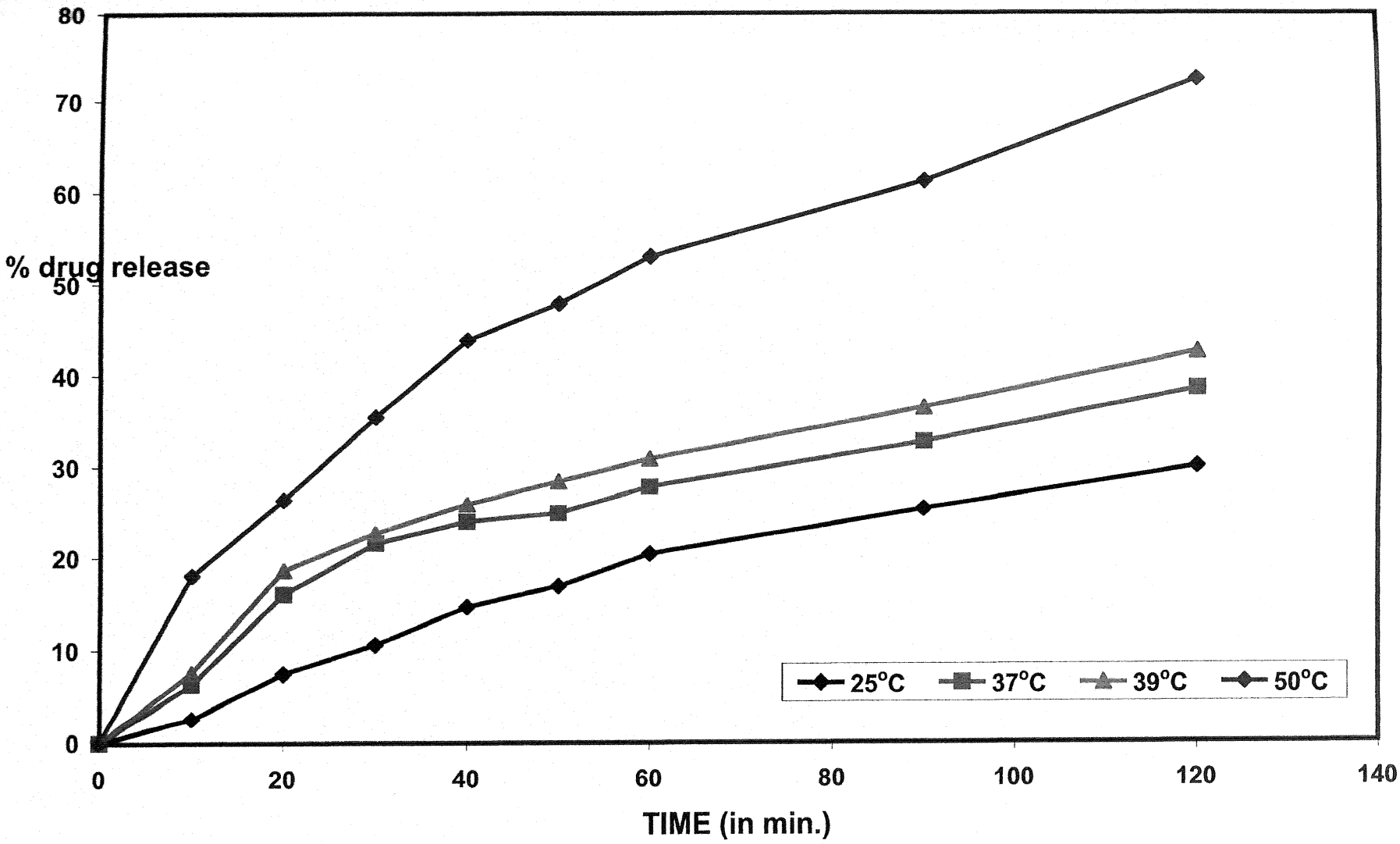


Figure-50: Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

Table-22 : Percentage drug release from 25^{0}C, 37^{0}C, 39^{0}C, 50^{0}C.

Model		Percentage release from different pH			
		25°C	37°C	39°C	50°C
Zero Order	R	0.9726	0.8665	0.8762	0.8782
	k	0.1503	0.2102	0.2348	0.3912
	SSQ	13	87	102	270
1st Order	R	0.9801	0.8958	0.9084	0.9343
	k	-0.0016	-0.0023	-0.0026	-0.0047
	SSQ	09	67	74	144
Matrix	R	0.9547	0.9872	0.9886	0.9942
	k	1.2965	1.8737	2.0898	3.4820
	SSQ	20	09	10	14
Peppas	R	0.9811	0.9590	0.9621	0.9948
	k	0.1658	0.8365	0.9415	2.3538
	SSQ	17	21	22	12
Hix Crow	R	0.9777	0.8865	0.8983	0.9179
	k	-0.0005	-0.0007	-0.0008	-0.0015
	SSQ	10	73	82	179
		PEPPAS	MATRIX	MATRIX	PEPPAS

Table-23: Percentage drug release from 25^{0}C.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	13	9	20	17	10
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	1.269	0.055	0.107	8.016	0.138	0.087
3.	20	3.747	0.548	0.336	4.208	0.228	0.402
4.	30	5.367	0.735	0.427	3.009	0.223	0.522
5.	40	7.529	2.300	1.678	0.450	1.024	1.876
6.	50	8.790	1.623	1.121	0.143	0.424	1.278
7.	60	10.725	2.910	2.313	0.465	0.935	2.504
8.	90	13.357	0.029	0.016	1.118	1.567	0.019
9.	120	15.958	4.324	2.567	3.082	12.213	3.068

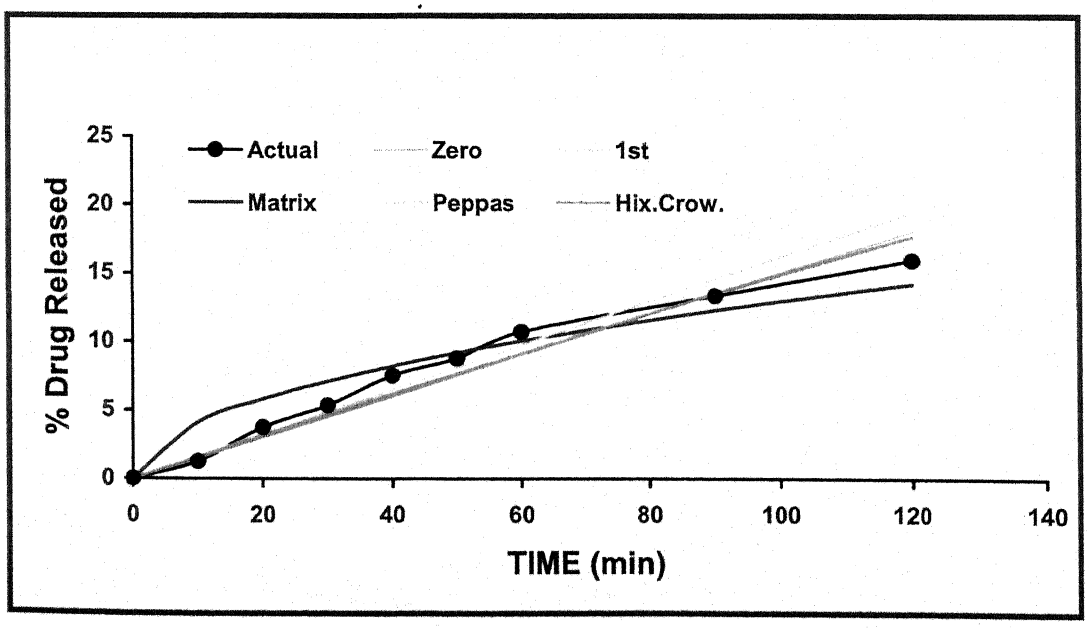


Figure-51: Percentage drug release from 25^{0}C.

Table-24: Percentage drug release from 37^{0}C.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	87	67	9	21	73
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	3.139	1.077	0.742	7.760	1.091	0.850
3.	20	8.099	15.178	12.923	0.078	1.708	13.672
4.	30	10.990	21.944	18.573	0.529	3.886	19.681
5.	40	12.369	15.698	12.695	0.269	1.800	13.665
6.	50	13.130	6.868	5.044	0.014	0.058	5.618
7.	60	14.884	5.168	3.880	0.137	0.059	4.284
8.	90	17.552	1.862	1.393	0.050	3.565	1.531
9.	120	20.789	19.649	11.369	0.070	8.889	13.656

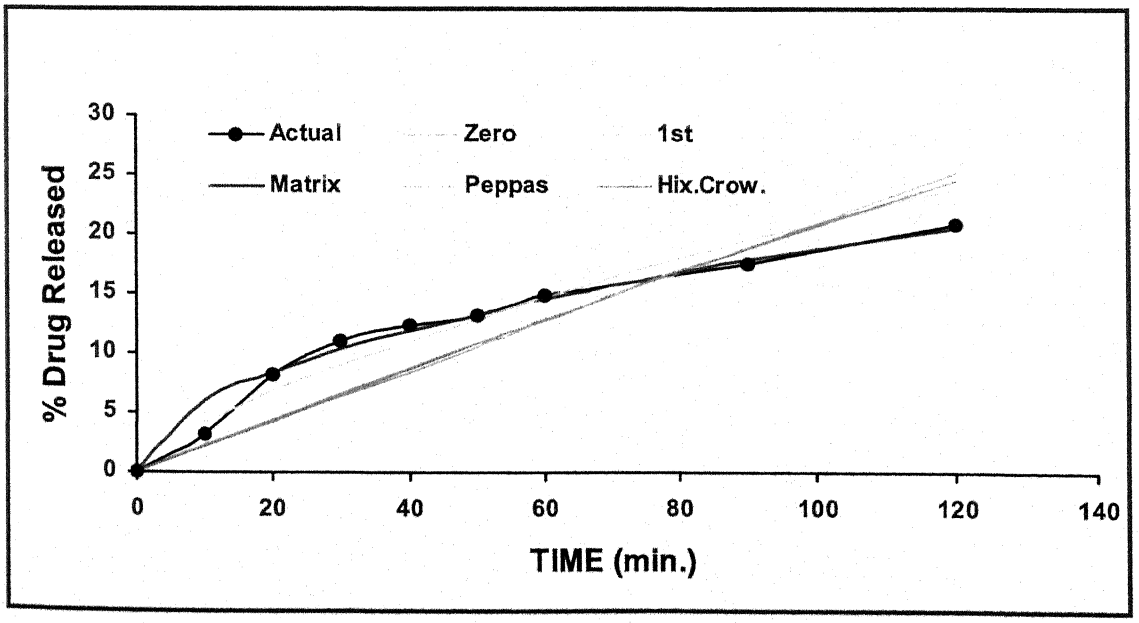


Figure-52: Percentage drug release from 37^{0}C.

Table-25 : Percentage drug release from 39^{0}C.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	102	74	10	22	82
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	3.541	1.423	0.937	9.412	1.299	1.094
3.	20	9.392	22.052	18.600	0.002	3.265	19.749
4.	30	11.545	20.261	16.194	0.010	2.205	17.521
5.	40	13.537	17.182	13.227	0.102	1.550	14.496
6.	50	14.922	10.126	7.312	0.021	0.317	8.195
7.	60	16.491	5.771	4.055	0.092	0.035	4.587
8.	90	19.630	2.258	1.637	0.038	3.974	1.817
9.	120	23.388	22.935	12.042	0.245	9.198	14.957

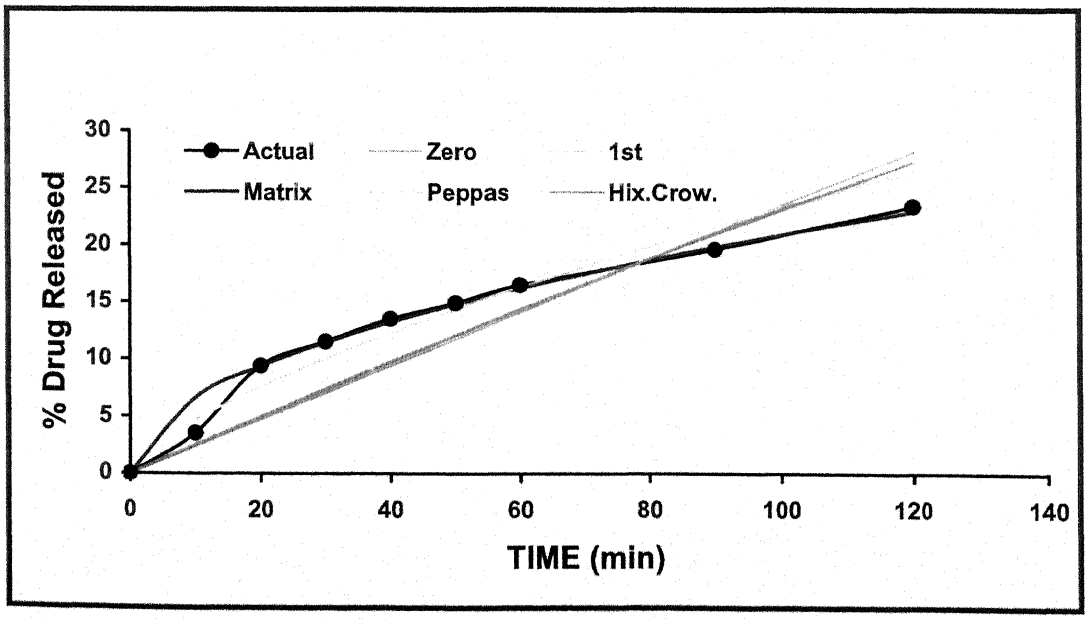


Figure-53 : Percentage drug release from 39^{0}C

Table-26: Percentage drug release from 50^{0}C.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	270	144	14	12	179
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	9.040	26.299	19.621	3.884	0.053	21.894
3.	20	13.356	30.601	18.895	4.912	0.421	22.678
4.	30	18.095	40.439	23.920	0.954	0.072	29.115
5.	40	22.683	49.494	29.997	0.437	2.328	36.035
6.	50	25.148	31.227	17.011	0.277	0.970	21.251
7.	60	28.215	22.502	12.606	1.547	1.644	15.526
8.	90	32.298	8.466	5.356	0.541	3.951	6.158
9.	120	39.141	60.875	16.851	0.995	2.399	26.250

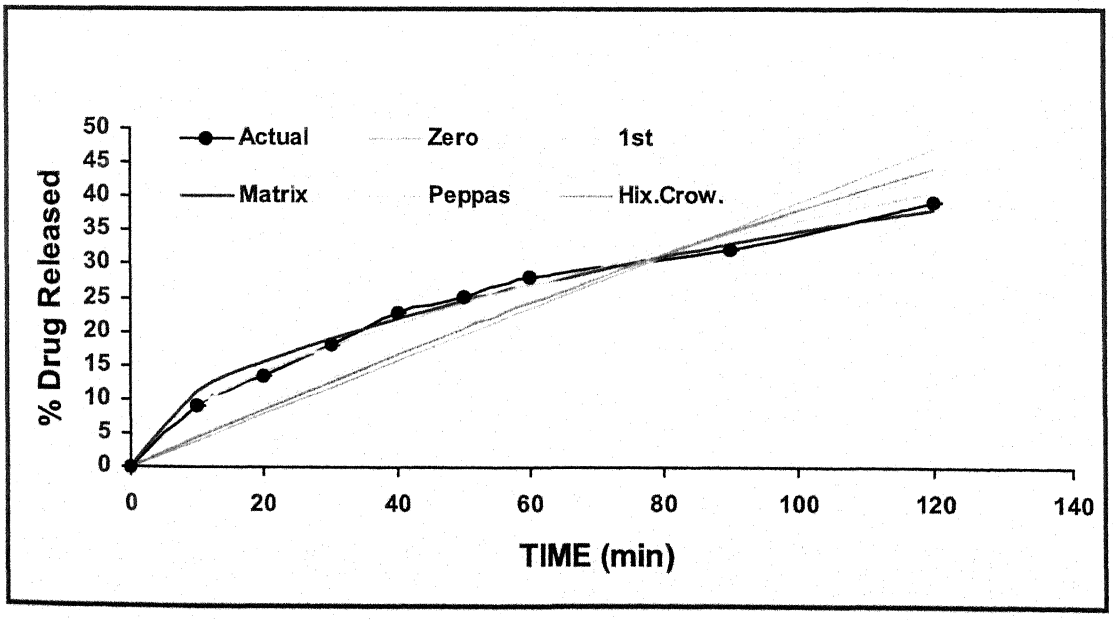


Figure-54: Percentage drug release from 50^{0}C.

6.2.3 Effect of stirring speed on the release of drug from liposomes :

The drug release test is conducted using a beaker with a magnetic stirrer. How ever for the liposomes, there is no detailed test in the official books. The stirring speed is varied based on the characteristic of drugs and the testing devices. The effect of stirring speed on the zidovudine release from liposomes is shown in fig-55 and table-27. It was shown in figure and table that increased stirring speed increase the rate of drug release. In addition with an increased stirring speed, the equilibrium time for drug release from conventional liposome decreased gradually. If a constant speed applied to the release device, the diffusion layer becomes thinner and drug release rate become larger. An increase in speed leads to reduction in the thickness of the diffusion layer. When the stirring speed 200 or more than two hundred and the dialysis bag may also be destroyed. Therefore an optimal stirring speed was 100rpm.

Table-27: Percentage release of drug at 50,100,150,200 rpm

S.No	Time in minutes	% Drug released at 50 rpm	% Drug released at 100 rpm	% Drug released at 150 rpm	% Drug released at 200 rpm
1.	0	0	0	0	0
2.	1	2.53	6.26	10.21	24
3.	2	5.64	16.73	18.45	33.3
4.	3	9.68	21.52	22.48	38.7
5.	4	13.25	23.79	26.79	43.1
6.	5	15.64	24.89	29.62	45.8
7.	6	18.72	27.26	32.14	49.4
8.	7	22.02	31.62	36.54	54.8
9.	8	26.58	38.54	45.23	62.9

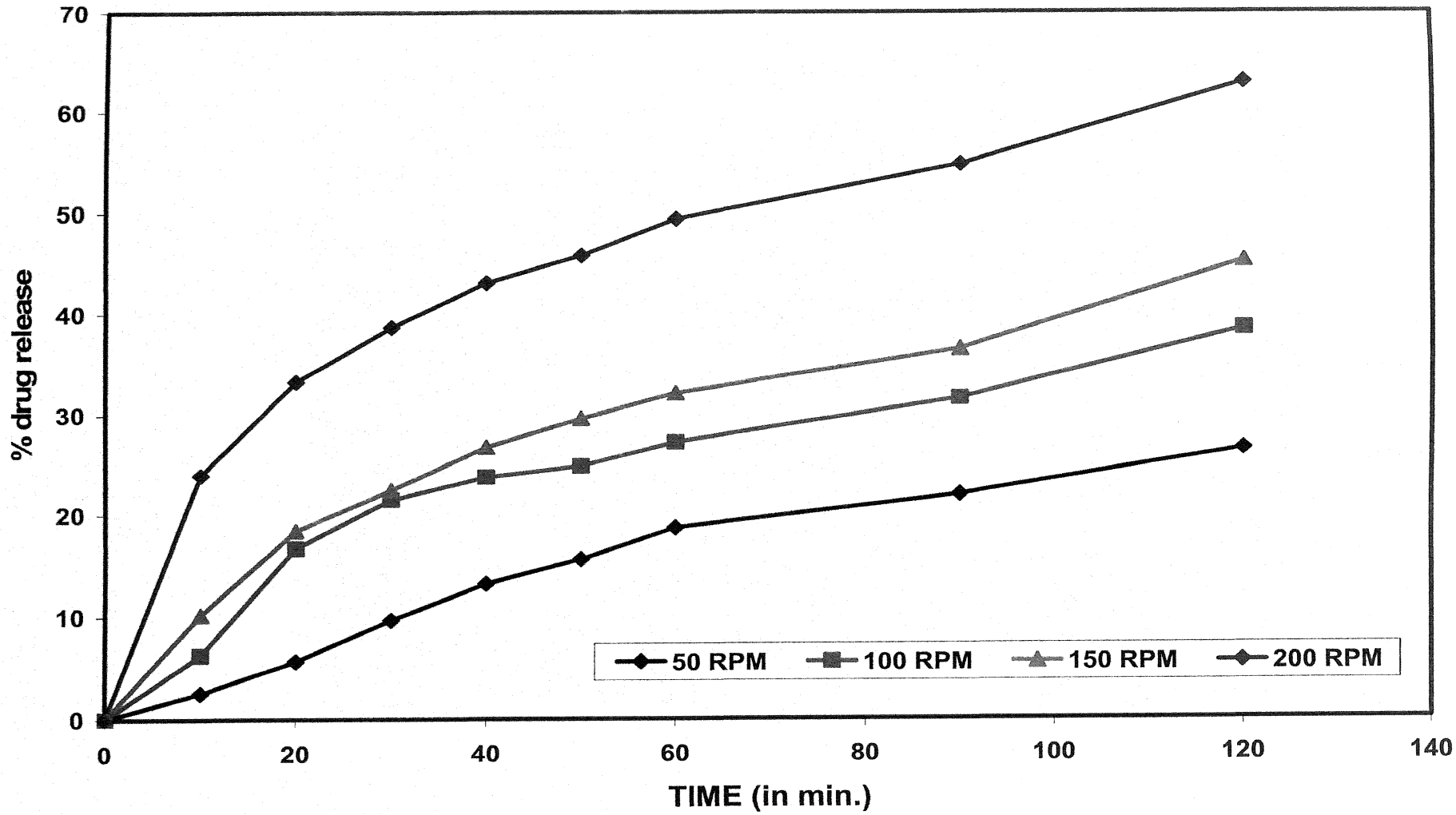


Figure-55: Percentage release of drug at 50,100,150,200 rpm

Table-28 : Percentage drug release from 50rpm, 100rpm, 150rpm, 200rpm.

Model		Percentage release from different rpm			
		50rpm	100rpm	150rpm	200rpm
Zero Order	R	0.9694	0.8595	0.8711	0.6623
	k	0.1334	0.2083	0.2422	0.3663
	SSQ	11	89	108	469
1st Order	R	0.9759	0.8899	0.9071	0.7737
	k	-0.0014	-0.0023	-0.0027	0.0043
	SSQ	08	69	78	325
Matrix	R	0.9516	0,9870	0.9946	0.9872
	k	1.1508	1.8590	2.1581	3.3411
	SSQ	17	09	05	21
Peppas	R	0.9858	0.9546	0.9901	0.9980
	k	0.1518	0.8804	1.4322	4.9414
	SSQ	13	20	06	02
Hix Crow	R	0.9739	0.8803	0.8959	0.7401
	k	-0.0005	-0.0007	-0.0009	-0.0014
	SSQ	09	75	87	367
		PEPPAS	MATRIX	MATRIX	PEPPAS

Table-29 : Percentage drug release from 50rpm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	11	8	17	13	9
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	1.253	0.007	0.024	5.695	0.048	0.017
3.	20	2.843	0.031	0.002	5.305	0.006	0.008
4.	30	4.907	0.817	0.556	1.950	0.307	0.638
5.	40	6.408	1.148	0.808	0.757	0.392	0.915
6.	50	8.138	2.153	1.689	0.000	0.870	1.837
7.	60	9.821	3.295	2.793	0.822	1.428	2.955
8.	90	11.694	0.099	0.077	0.603	1.380	0.084
9.	120	14.123	3.566	2.287	2.298	8.820	2.660

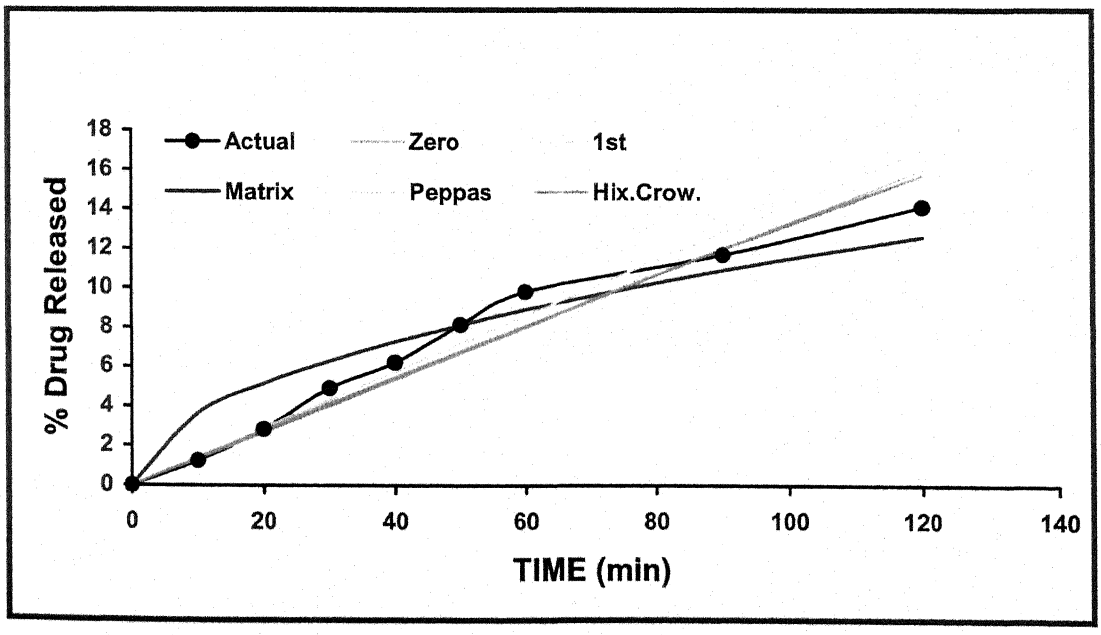


Figure-56 : Percentage drug release from 50rpm

Table-30 : Percentage drug release from 100rpm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	89	69	9	20	75
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	3.159	1.159	0.816	7.393	1.213	0.927
3.	20	8.421	18.104	15.678	0.012	2.469	16.486
4.	30	10.957	22.159	18.831	0.600	3.666	19.926
5.	40	12.255	15.387	12.467	0.248	1.547	13.411
6.	50	13.133	7.388	5.523	0.000	0.093	6.112
7.	60	14.587	4.361	3.206	0.035	0.003	3.567
8.	90	17.068	2.822	2.247	0.323	4.493	2.418
9.	120	20.797	17.639	9.974	0.188	6.598	12.083

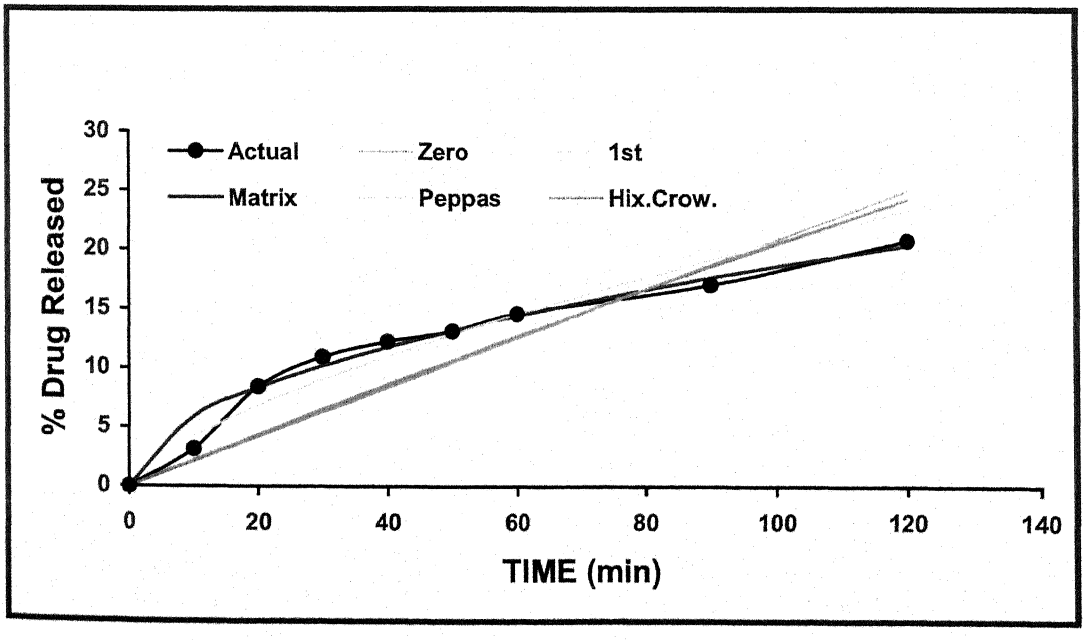


Figure-57: Percentage drug release from 100rpm

Table-31 : Percentage drug release from 150rpm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	108	78	5	6	87
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	5.106	7.208	5.975	2.952	0.365	6.389
3.	20	9.323	20.070	16.563	0.108	0.439	17.728
4.	30	11.535	18.233	14.131	0.082	0.236	15.464
5.	40	13.867	17.480	13.224	0.048	0.539	14.586
6.	50	15.619	12.333	8.995	0.129	0.364	10.045
7.	60	17.140	6.819	4.815	0.180	0.149	5.437
8.	90	19.670	4.511	3.572	0.646	2.908	3.846
9.	120	24.451	21.229	10.291	0.657	0.915	13.160

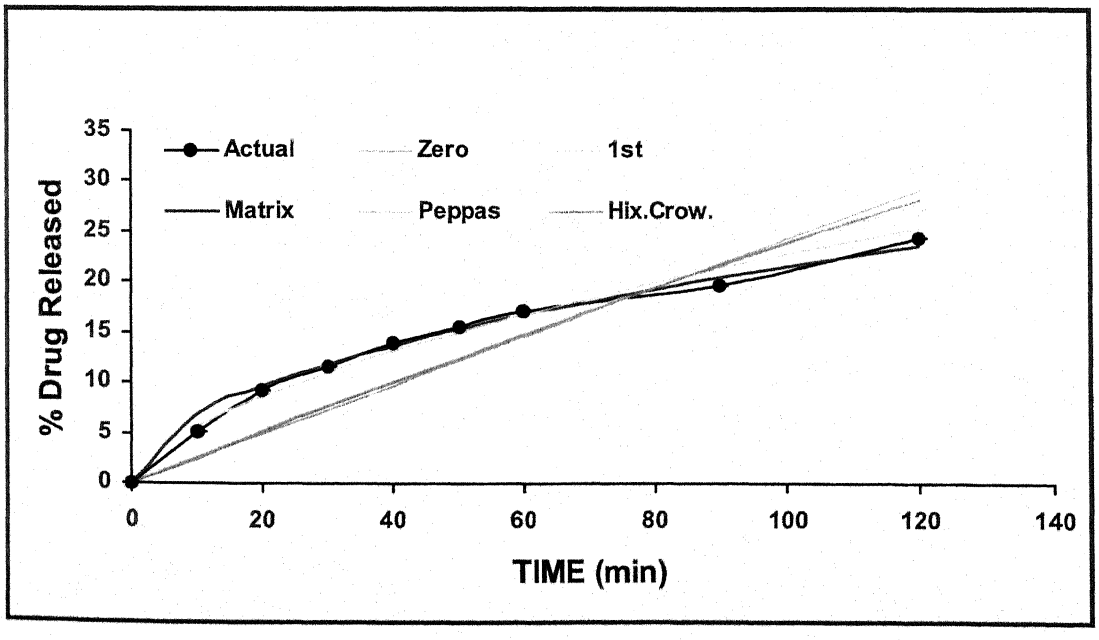


Figure-58: Percentage drug release from 150rpm

Table-32 : Percentage drug release from 200rpm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	469	325	21	2	367
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	12.252	73.755	64.272	2.843	0.121	67.550
3.	20	16.912	91.877	74.344	3.882	0.045	80.222
4.	30	19.935	80.006	60.243	2.673	0.059	66.669
5.	40	22.650	63.944	45.669	2.308	0.266	51.450
6.	50	24.288	35.652	23.369	0.440	0.003	27.130
7.	60	26.553	20.900	13.598	0.452	0.204	15.796
8.	90	29.816	9.955	5.947	3.536	0.925	7.019
9.	120	34.340	92.587	37.974	5.110	0.066	50.887

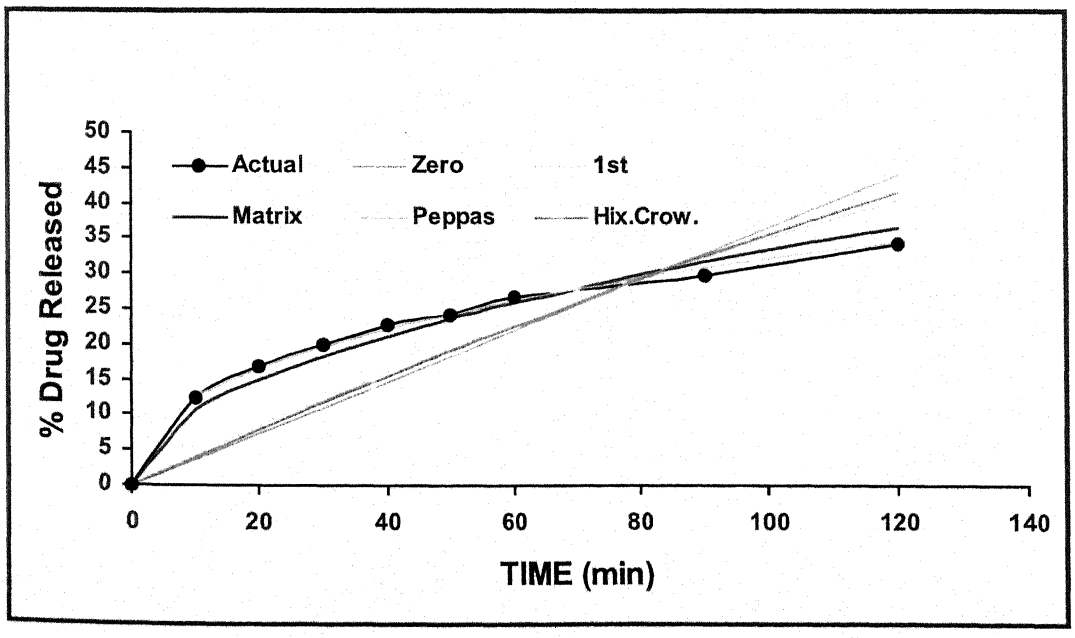


Figure-59: Percentage drug release from 200rpm

6.2.4 Effect of liposome mean size in the release kinetics.

The zidovudine release from the conventional liposomes of different size is shown in Fig-60 and Table-33. The data was fitted with the Highuchi law and showed a good application of this model to zidovudine release from conventional liposomes. The surface area of liposomes exposed to the release medium is part of the release. The released amount of zidovudine is related to the size/surface area of liposomes. The zidovudine release rate increased with decreasing liposome size because of the surface area of liposomes was increased. Increasing the surface area of the increased total surface area exposed to the release medium increased the amount of zidovudine diffusion from liposomes.

Table-33: Zidovudine release percentage from conventional liposomes with the mean diameter of 601nm, 767nm, 995nm, 1110nm (stirring speed 100 rpm).

S. No.	Time in minutes	Mean particle size601nm	Mean particle size767nm	Mean particle size995nm	Mean particle size1110nm
0.	0	0	0	0	0
1.	10	4.52	4.85	8.54	14.7
2.	20	5.12	7.58	14.21	23
3.	30	8.56	10.75	20.6	30.9
4.	40	10.47	14.82	25	36.8
5.	50	12.53	18.59	28.4	40.7
6.	60	15.64	21.34	33.45	44.87

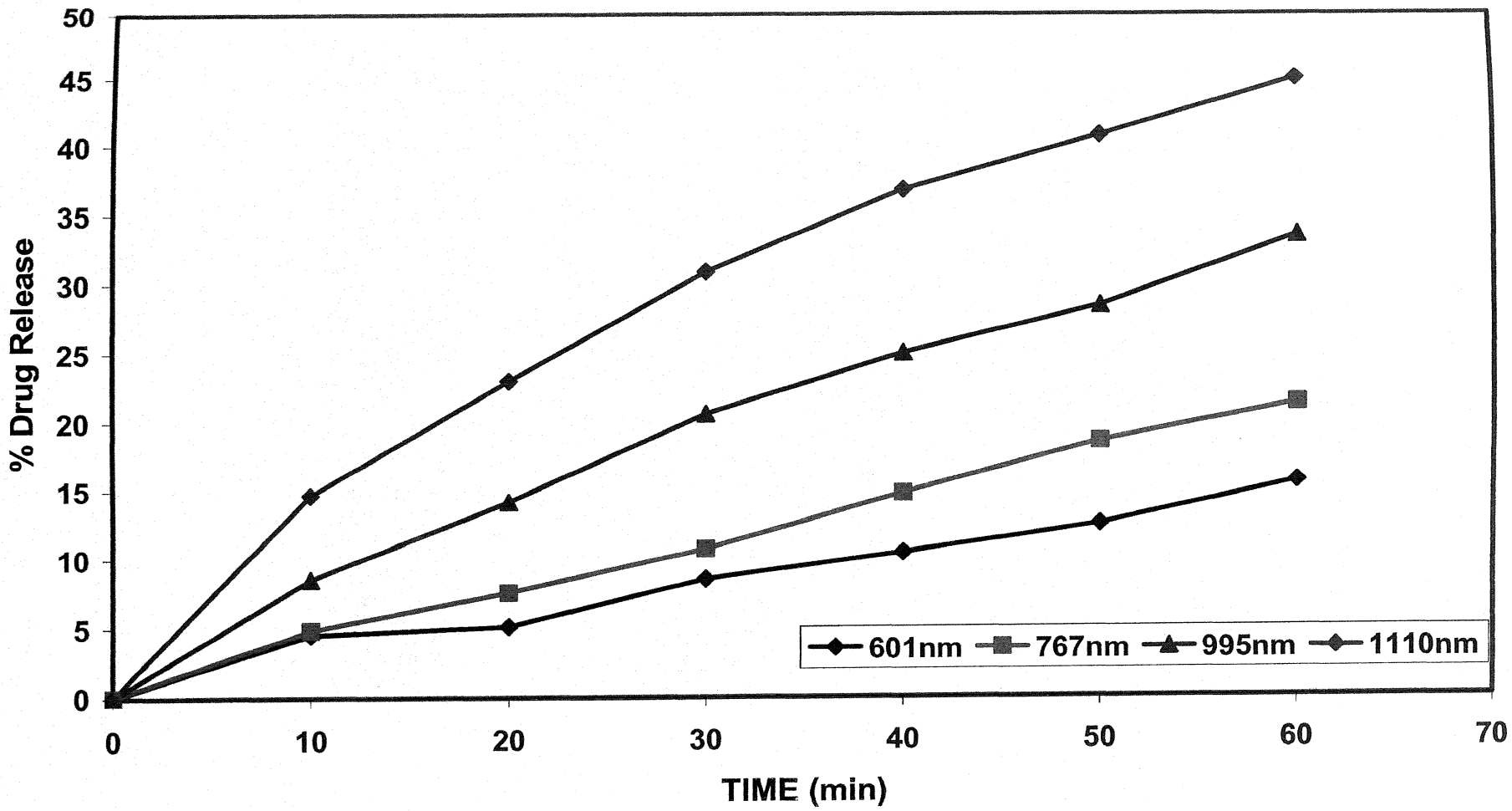


Figure-60: Zidovudine release percentage from conventional liposomes with the mean diameter of 601nm, 767nm, 995nm, 1110nm (stirring speed 100 rpm).

Table-34 : Percentage drug release from 601nm, 767nm, 995nm, 1110nm.

Model		Percentage release on different particle size.			
		601nm	767nm	995nm	1110nm
Zero Order	R	0.9889	0.9980	0.9886	0.9613
	k	0.1367	1.902	0.3109	0.4451
	SSQ	01	0	05	32
1st Order	R	0.9900	0.9983	0.9934	0.9755
	k	-0.0014	-0.0020	-0.0034	-0.0050
	SSQ	01	0	03	20
Matrix	R	0.9527	0.9454	0.9722	0.9906
	k	0.8914	1.2333	2.0380	2.9497
	SSQ	04	10	13	08
Peppas	R	0.9635	0.9944	0.9991	0.9989
	k	0.3565	0.3004	0.6822	1.6055
	SSQ	01	01	0	01
Hix Crow	R	0.9896	0.9983	0.9920	0.9711
	k	-0.0005	-0.0007	-0.0011	-0.0016
	SSQ	01	0	04	24
		IST ORDER	IST ORDER	PEPPAS	PEPPAS

Table-35 : Percentage drug release from 601nm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	1	1	4	1	1
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	2.296	0.863	0.796	0.273	0.109	0.818
3.	20	2.583	0.023	0.042	1.969	0.496	0.035
4.	30	4.380	0.077	0.051	0.252	0.004	0.059
5.	40	5.419	0.003	0.007	0.048	0.006	0.005
6.	50	6.558	0.078	0.073	0.065	0.005	0.075
7.	60	8.228	0.001	0.008	1.753	0.646	0.005

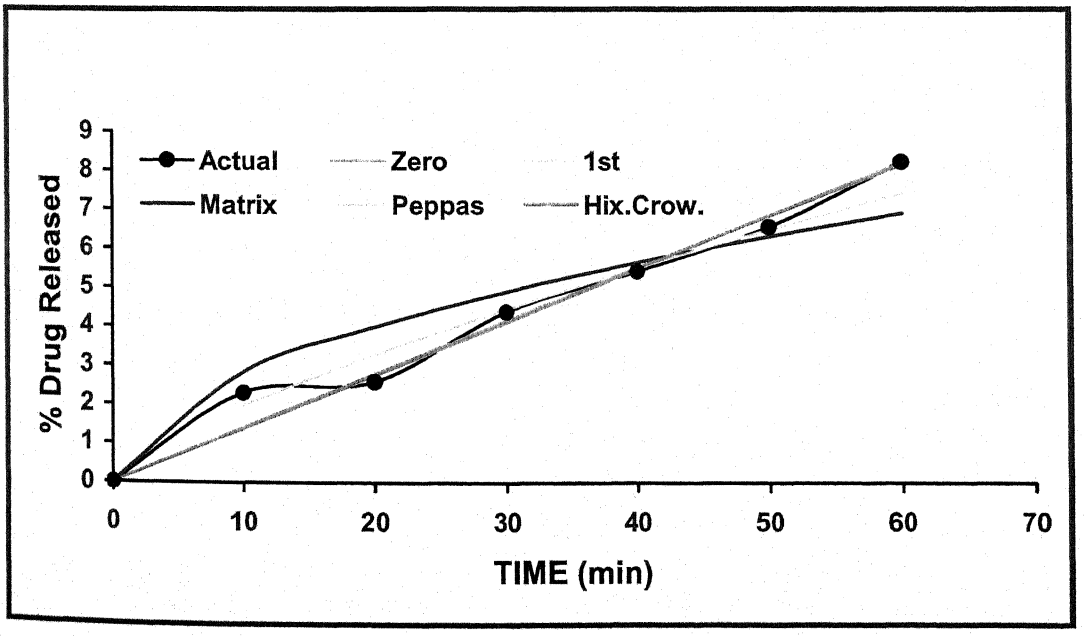


Figure-61: Percentage drug release from 601nm

Table-36: Percentage drug release from 767nm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	0	0	10	1	0
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	2.425	0.273	0.202	2.176	0.027	0.224
3.	20	3.850	0.002	0.004	2.773	0.092	0.001
4.	30	5.500	0.043	0.097	1.575	0.181	0.077
5.	40	7.667	0.003	0.000	0.018	0.002	0.000
6.	50	9.676	0.027	0.032	0.913	0.162	0.030
7.	60	11.241	0.030	0.002	2.849	0.130	0.007

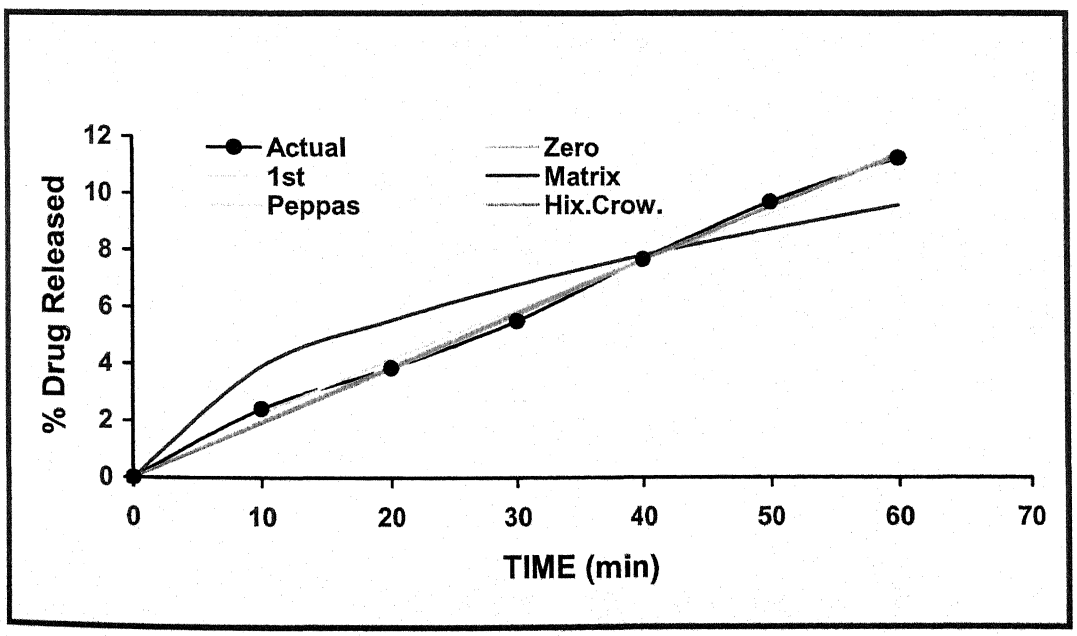


Figure-62: Percentage drug release from 767nm

Table-37: Percentage drug release from 995nm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	5	3	13	0	4
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	4.263	1.332	0.917	4.758	0.000	1.050
3.	20	7.199	0.961	0.485	3.669	0.033	0.627
4.	30	10.532	1.451	0.882	0.397	0.119	1.055
5.	40	12.946	0.260	0.133	0.003	0.020	0.170
6.	50	14.943	0.364	0.280	0.283	0.120	0.305
7.	60	17.696	0.919	0.325	3.649	0.001	0.478

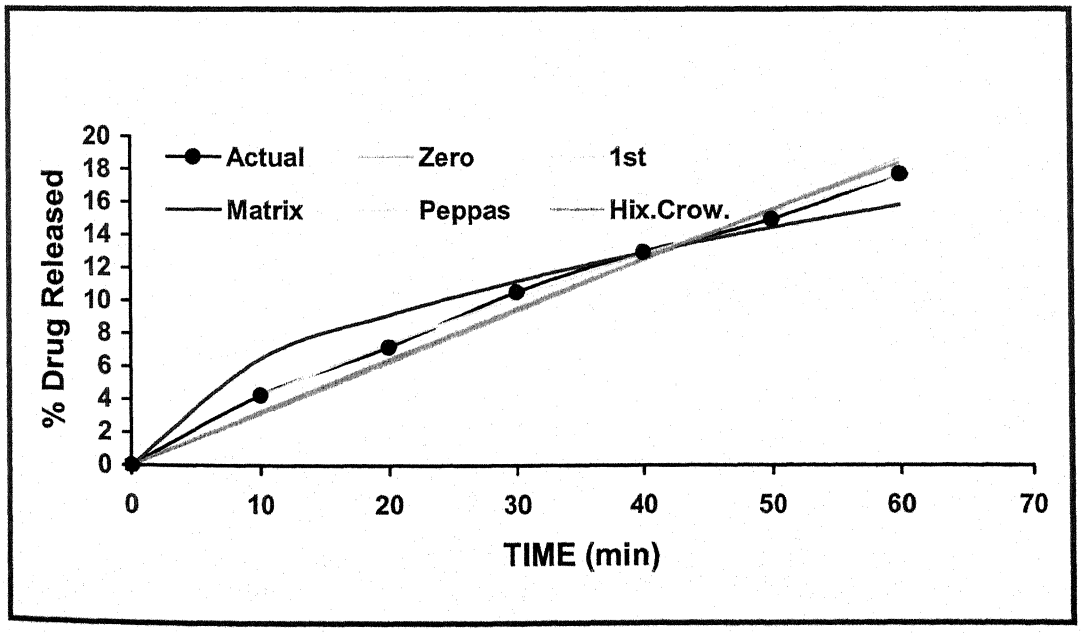


Figure-63: Percentage drug release from 995nm

Table-38: Percentage drug release from 1110nm.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	32	20	8	1	24
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	7.354	8.430	6.227	3.895	0.005	6.946
3.	20	11.656	7.586	4.727	2.358	0.012	5.615
4.	30	15.840	6.188	3.842	0.100	0.187	4.555
5.	40	19.100	1.681	1.072	0.197	0.198	1.255
6.	50	21.397	0.735	0.421	0.291	0.059	0.508
7.	60	23.909	7.817	3.703	1.125	0.269	4.793

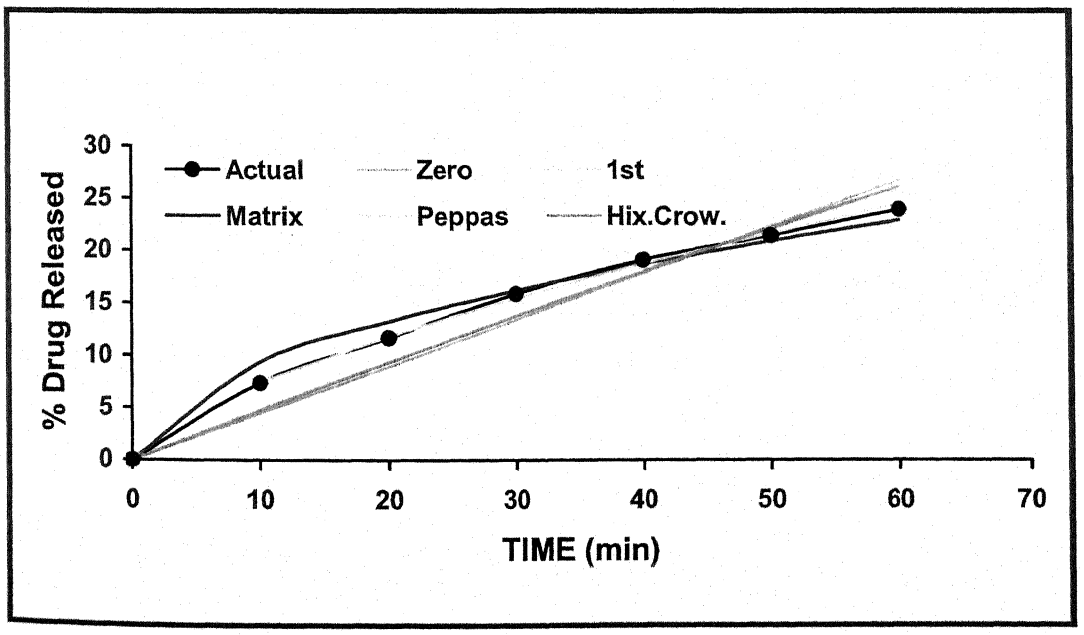


Figure-64: Percentage drug release from 1110nm

6.2.5 Effect of pH on the liposome release kinetics.

Zidovudine release from conventional liposomes as a function of pH is shown in fig-65 and table39. The Zidovudine release from liposome was pH dependent. The release results shown in figure indicated that pH had significant effect on the Zidovudine release from conventional liposomes. The release studies were conducted in different pH ranges i.e. 3.0, 5.0, 7.0, 8.0. PH range from 3-5 increased the Zidovudine release rate from conventional liposomes. PH 3.0 Zidovudine released the drug substance very faster than 7.0 and 8.0. So, there is a slight variation in the drug release. The faster release in pH –3.0 may be due to fusion of the liposomes.

Table-39 : Percentage release as a function of pH-3.0, 5.0, 7.0, 8.0.

S. No.	Time in minutes	pH 8.0	pH 7.0	pH 5.0	pH 3.0
1.	10	5.5	6.8	12	17.3
2.	20	10	12.7	17.6	22.4
3.	30	12	14.8	21.2	27.3
4.	40	14	19.4	25.2	30.7
5.	50	18.4	21.2	28.5	32.7
6.	60	21.2	23.6	30	34.8

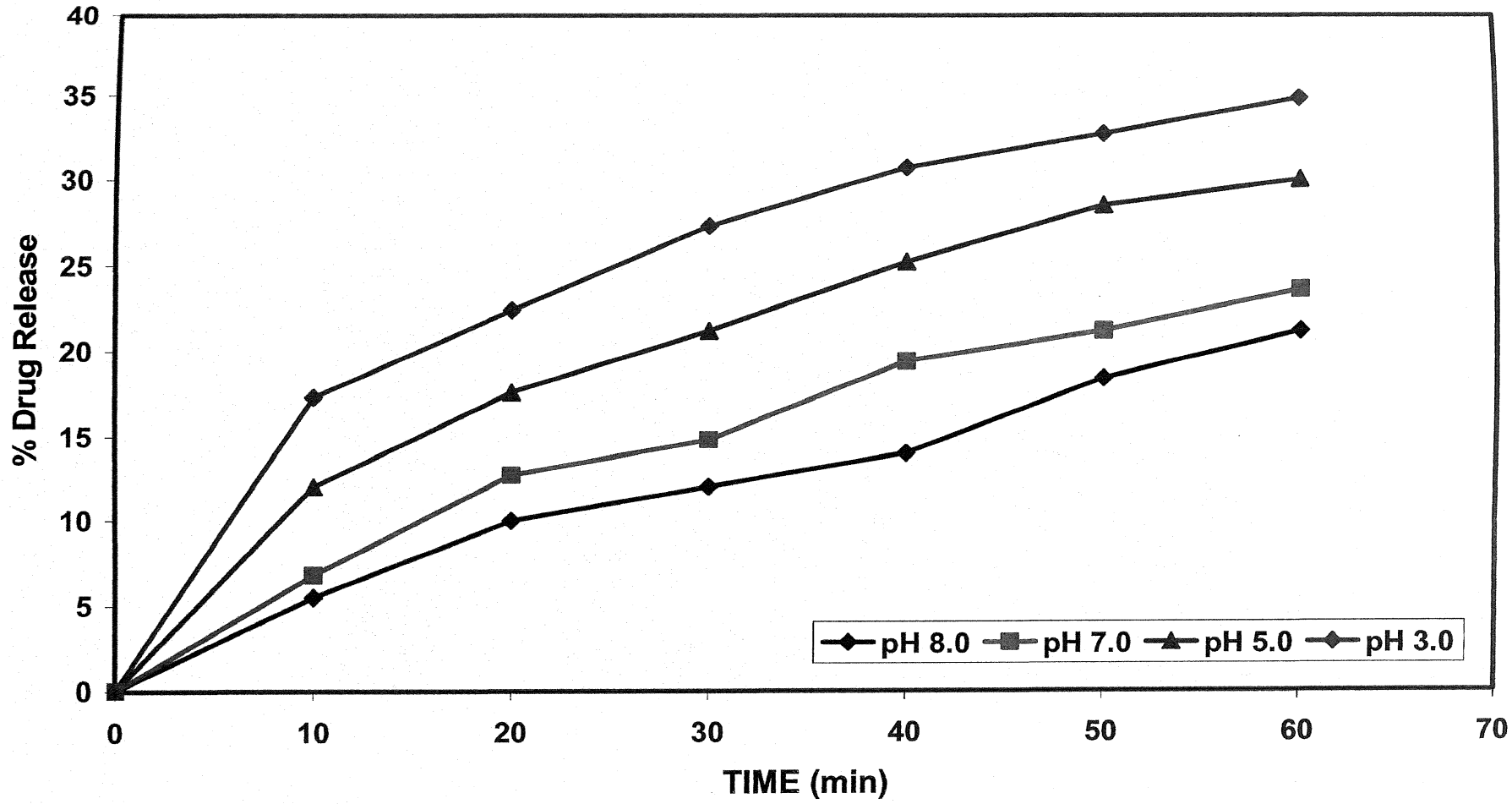


Figure-65: Percentage release as a function of pH-3.0, 5.0, 7.0, 8.0.

Table-40: Percentage drug release from pH-8, pH-7, pH-5, pH-3.

Model		Percentage release from different pH			
		pH-8.0	pH-7.0	pH-5.0	pH-3.0
Zero Order	R	0.9856	0.9663	0.9291	0.8616
	k	0.1935	0.2726	0.3083	0.3700
	SSQ	03	15	26	64
1st Order	R	0.9885	0.9594	0.9431	0.8857
	k	0.0020	-0.0029	-0.0033	-0.0040
	SSQ	02	16	21	01
Matrix	R	0.9687	0.9025	0.9982	0.9973
	k	1.2682	1.7598	2.0589	2.4965
	SSQ	06	41	01	01
Peppas	R	0.9935	0.9726	0.9993	0.9988
	k	0.4916	0.4720	1.6775	3.1374
	SSQ	01	18	0	0
Hix Crow	R	0.9876	0.9619	0.9386	0.8780
	k	-0.0007	-0.0010	-0.0011	-0.0013
	SSQ	02	15	22	56
		PEPPAS	PEPPAS	PEPPAS	PEPPAS

Table-41 : Percentage drug release from pH-5.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	26	21	1	0	22
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	6.010	8.564	7.522	0.251	0.000	7.869
3.	20	8.940	7.693	6.313	0.072	0.006	6.761
4.	30	10.942	2.868	2.127	0.112	0.025	2.360
5.	40	13.112	0.609	0.451	0.008	0.008	0.500
6.	50	15.020	0.156	0.079	0.213	0.077	0.101
7.	60	16.048	6.003	4.082	0.010	0.071	4.645

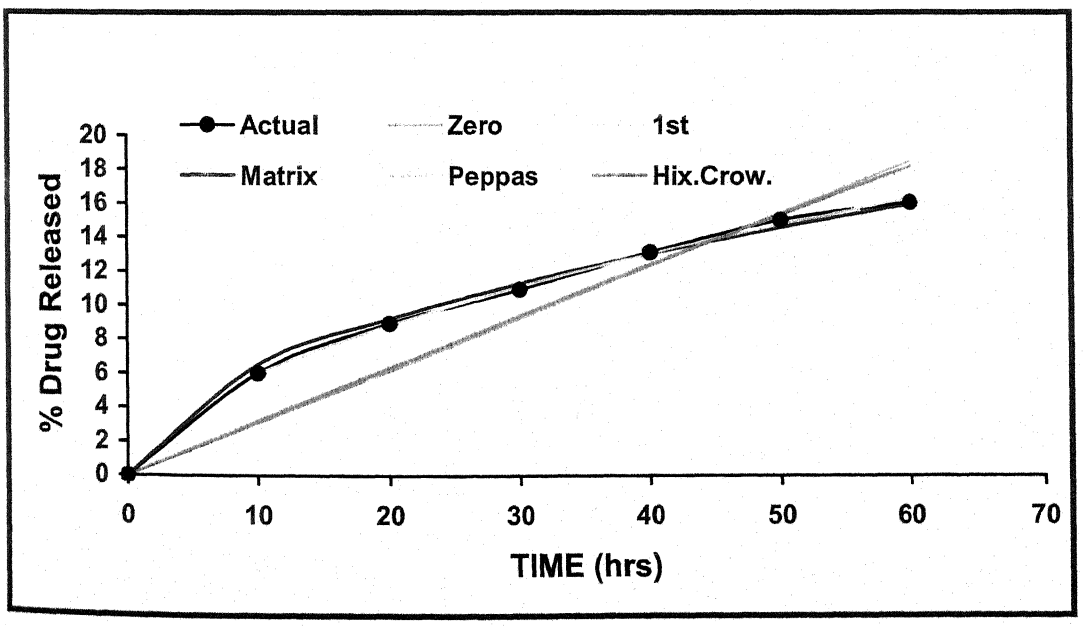


Figure-66: Percentage drug release from pH-5.

Table-42 : Percentage drug release from pH-3.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	64	52	1	0	56
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	8.659	24.588	22.037	0.584	0.004	22.891
3.	20	11.381	15.845	13.026	0.047	0.071	13.940
4.	30	14.054	8.721	6.893	0.144	0.020	7.472
5.	40	16.033	1.518	1.195	0.059	0.065	1.296
6.	50	17.344	1.340	0.937	0.095	0.003	1.056
7.	60	18.715	12.157	8.042	0.388	0.017	9.227

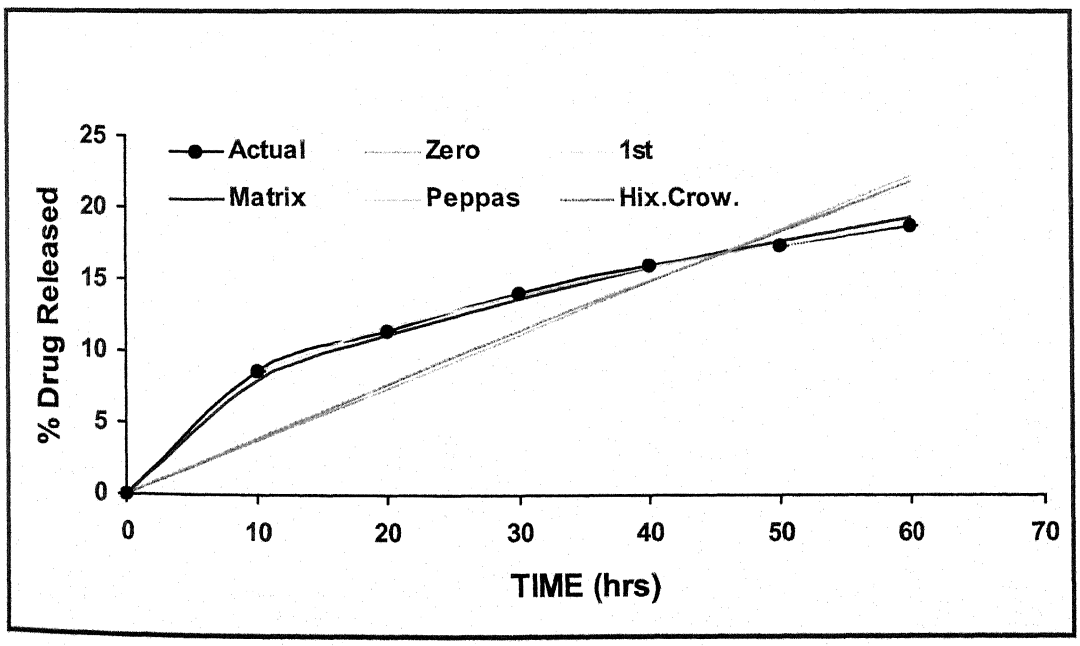


Figure-67 : Percentage drug release from pH-3.

Table-43 : Percentage drug release from pH-8.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	3	2	6	1	2
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	2.758	0.677	0.561	1.568	0.001	0.599
3.	20	5.059	1.414	1.170	0.375	0.122	1.249
4.	30	6.165	0.130	0.067	0.610	0.053	0.085
5.	40	7.288	0.204	0.258	0.536	0.432	0.240
6.	50	9.616	0.003	0.001	0.421	0.046	0.002
7.	60	11.246	0.133	0.048	2.023	0.209	0.070

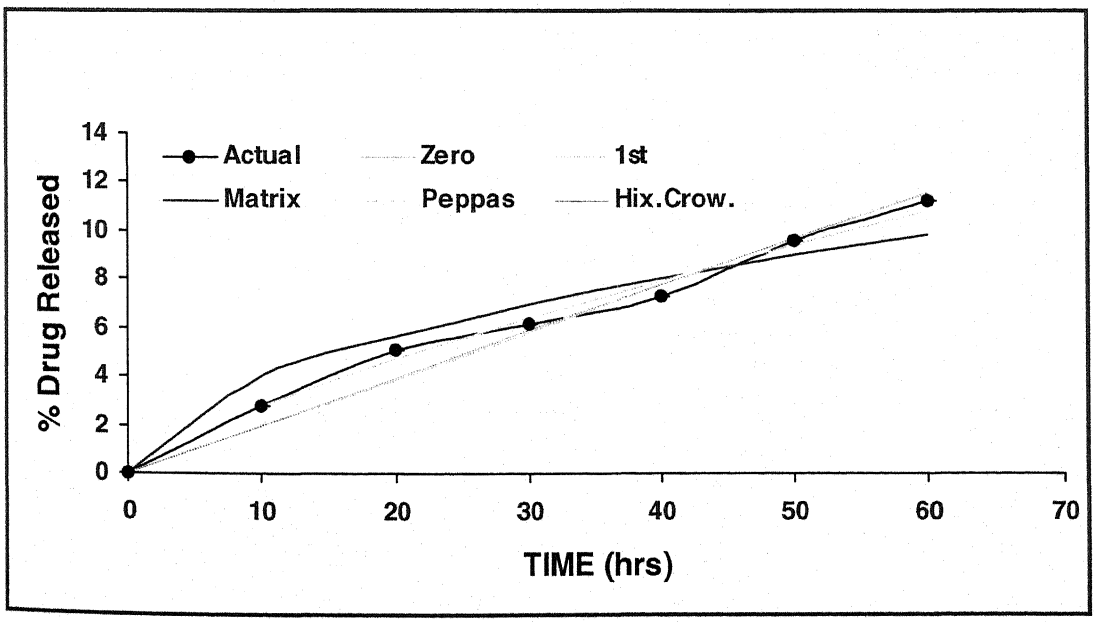


Figure-68: Percentage drug release from pH-8.

Table-44 : Percentage drug release from pH-7.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	15	16	41	18	15
1.	0	0.000	0.000	0.000	0.000	-	0.000
2.	10	3.400	0.454	0.256	4.685	0.007	0.318
3.	20	6.419	0.933	0.511	2.106	0.203	0.639
4.	30	7.609	0.324	0.678	4.117	0.648	0.545
5.	40	10.046	0.739	1.076	1.175	0.476	0.956
6.	50	11.183	5.996	6.115	1.588	3.191	6.069
7.	60	18.822	6.072	7.116	26.951	13.596	6.783

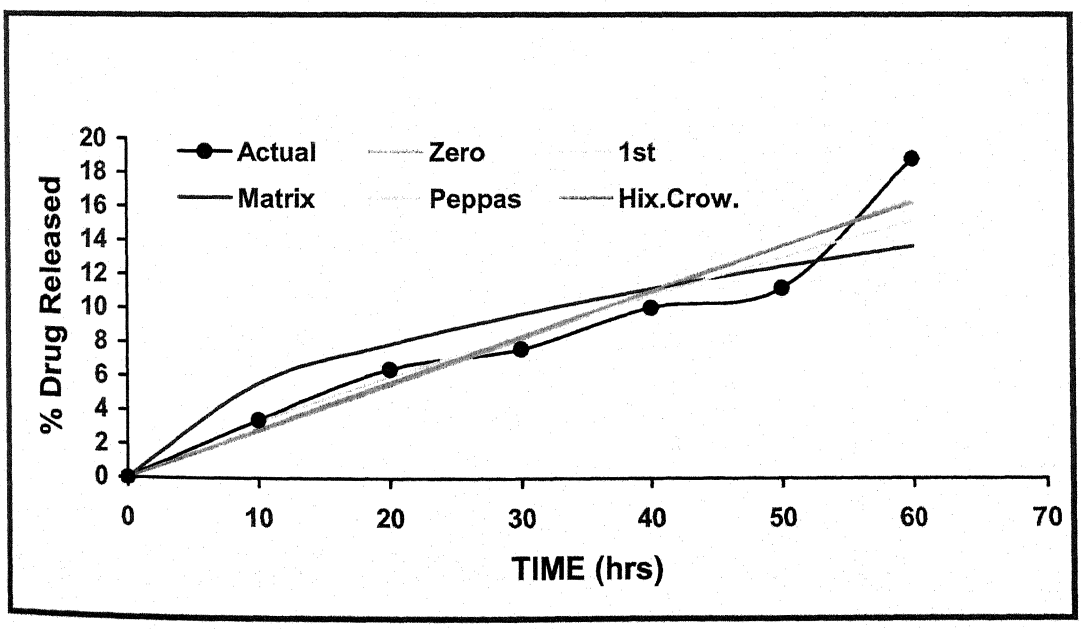


Figure-69: Percentage drug release from pH-7.

6.2.6 Effects of salts on stability (Optical microscope observation)

The minimum shelf life of any dosage form is at least one year for the solid dosage forms may vary from 1 year to up to 5 years and for the liquid dosage forms are also for around 3 years. But in the liposomes formulations, maintaining their physical and chemical properties is often difficult. Instability problem of the liposomes as a result of the combination of flocculation or aggregation or fusion or coalescence. To overcome this problems simply by increasing the electrostatic repulsion between liposomes such as with an increase of the charge on the liposome and decrease the ionic strength of the medium. The human body contains high salt concentration (Nacl, $Cacl_2$, and Kcl). The stability of liposomes remains high in this environment. The energy barrier between approaching liposomes is reduced by increasing ionic strength of the medium, thus facilitating effective collision that leads to the formation of vesicles doublets, triplets, and quadruplet. In addition to the repulsion can be reduced between liposomes by incorporation of stabilizers like polymers (Steric stabilization). The main advantage of the steric stabilization is that not pH and salt dependent. On the addition of salts like Nacl,Kcl,$Cacl_2$ with different concentration(0.001M, 0.01M,0.1M,1M) and various pH ranges from 3-8 to under stand the stability of the liposomes. The liposomes may under go the coalescence process, the fusion may occur and the size of the liposomes increases irreversibly.

On addition of salts to the colloidal liposomal preparation, the aggregation of liposomes are seemed as singlet, doublets, triplets, quadruplets and on high salt concentration the total mass aggregation occurred. These effects are observed under the optical microscope.

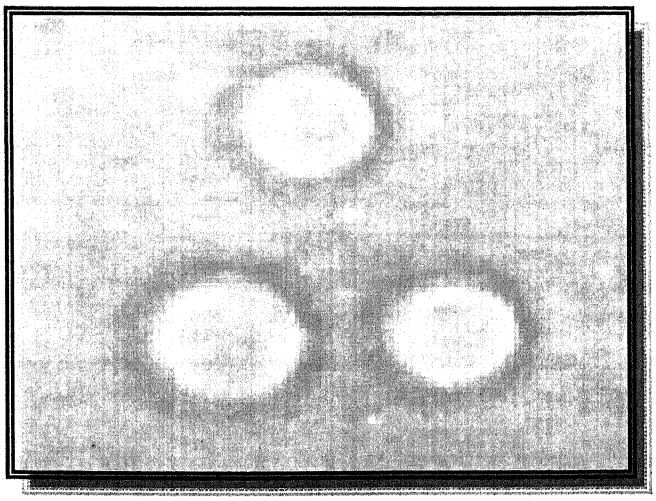


Figure-70: Particle size of the liposomes vesicle after one-month storage

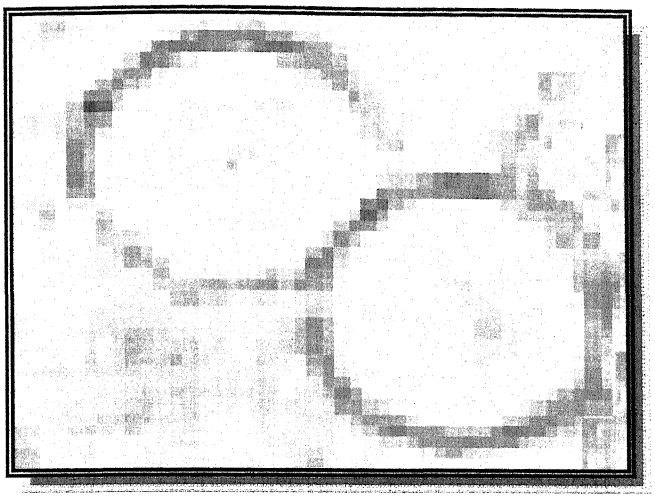


Figure-71: On addition of salt, pH- Liposomes come closer and form Doublet

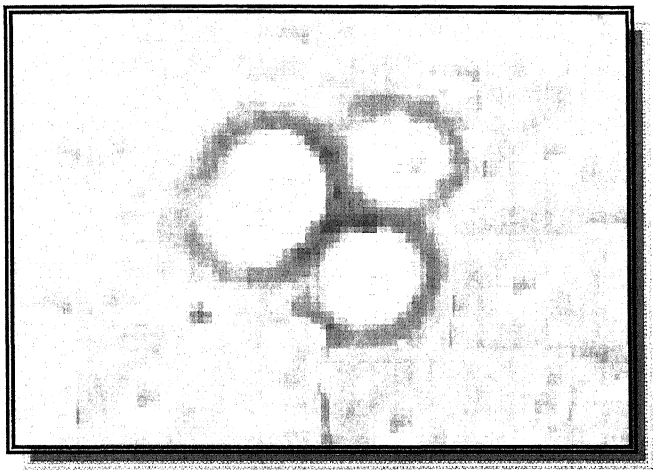


Figure-72: On addition of salt, pH- Liposomes come closer and form Triplet

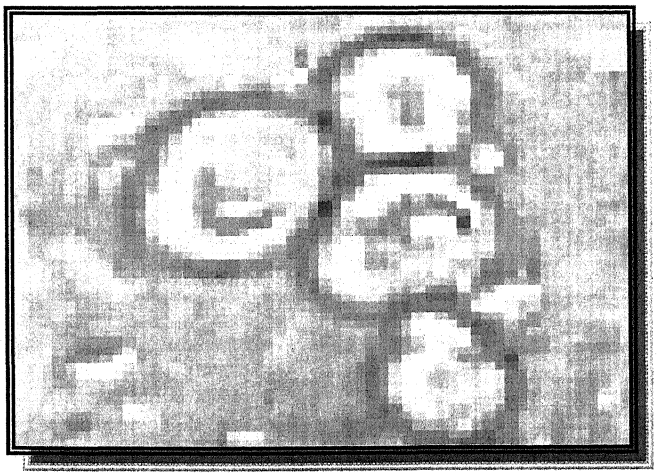


Figure-73: On addition of salt, pH- Liposomes come closer and form Quadruplet

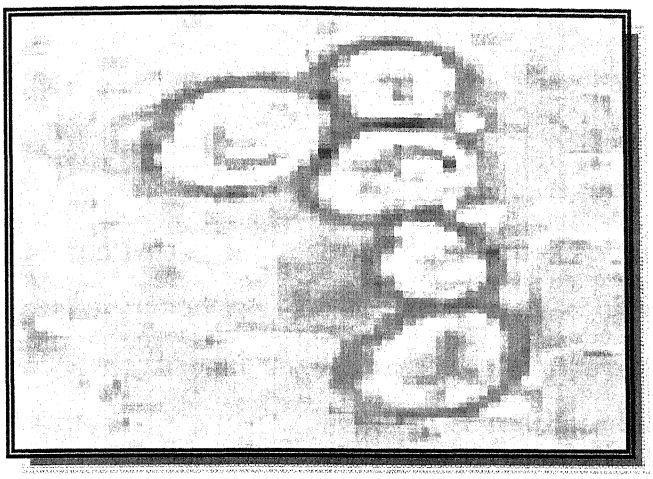


Figure-74: On addition of salt, pH- Liposomes come closer and start to form aggregates

Figure-75: On addition of salt, pH- Liposomes come closer and form aggregates

Figure-76: On higher concentration of salt, pH- Liposomes ruptures and breaks the vesicles

6.2.7 Effect of temperature on liposomes size enlargement:

The conventional liposomes containing zidovudine were taken and stored at different temperatures like 4°C, 25°C, 37°C, and 50°C for 30 days. The influence of temperature on liposomes diameter as a function of time have been investigated and are shown in the fig-77 and values of the size enlargement of liposomes shown in table–45. In the higher temperatures, the liposomes may aggregate and form clusters. This investigation is to find out the effects on size enlargement of liposomes and its suitable storage temperature. The liposomal coalescence is not reversible and it is sensitive to temperature. The aggregation of liposomes due to Brownian motion while increasing the temperature, there will be increasing in motion of the liposomes vesicles. The reaction limited cluster aggregation process, the average cluster size grows exponentially with time. After aggregation liposome tends to form large vesicles via coalescence. In this case on decreasing the temperature of zidovudine liposomes increased the viscosity of liposomal suspension and the liposomal coalescence rate decreased. So it is shown in this study that a low temperature i.e. 4°C increases the stability of liposomes in relation to their size.

Table-45 : Effect of temperature on liposome size enlargement.

S. No.	Time in days	4°C	25°C	37°C	50°C
1.	0	0	0	0	0
2.	5	2.458	2.498	2.575	4.13
3.	10	2.635	2.891	3.4	7.3
4.	15	2.896	3.17	3.94	11.4
5.	20	2.941	3.621	4.61	--
6.	25	3.01	4.15	5.28	--

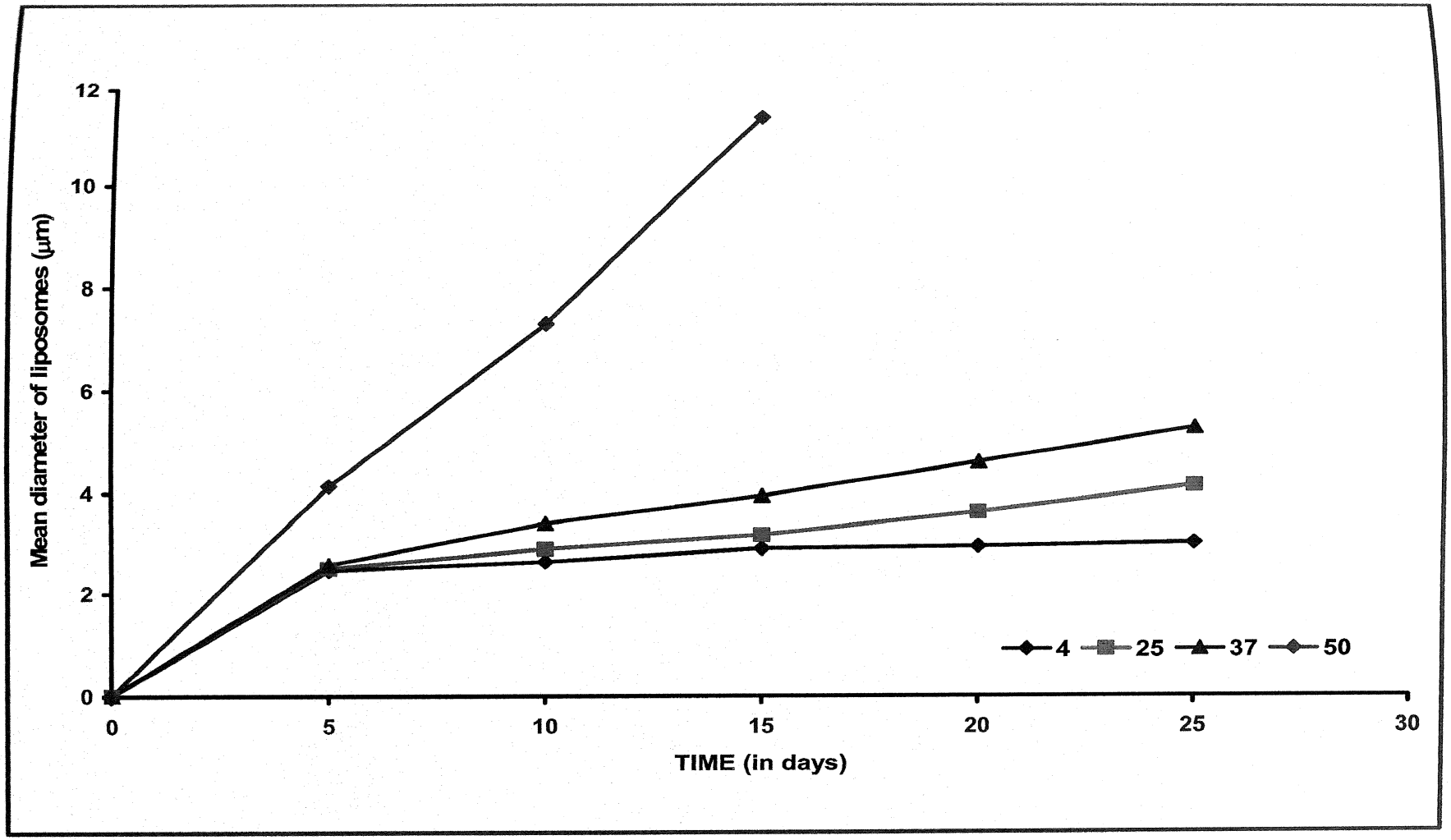


Figure-77: Effect of temperature on liposome size enlargement

6.2.8 Effect of pH on liposome size enlargement:

The conventional liposomes containing zidovudine were stored at different values like 3,5,7,8. The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-78 and values of the size enlargement of liposomes shown in table-46. It was found that the effect of pH on liposome stability is significant. The pH range above5-8, there is no significant change in the size enlargement. But the decreased pH (pH-3), the rate of membrane fusion may occur in the neutral liposomes.

Table-46: Effect of different pH on liposomes size enlargement.

S. No.	Time in days	Effect of size in pH-7	Effect of size in pH-8	Effect of size in pH-5	Effect of size in pH-3
1.	0	0	0	0	0
2.	5	2.54	2.88	3.53	4.52
3.	10	2.98	3.6	4.95	6.98
4.	15	3.37	4.07	6	8.56
5.	20	3.47	4.46	6.56	12.34
6.	25	3.6	5.06	7.7	--

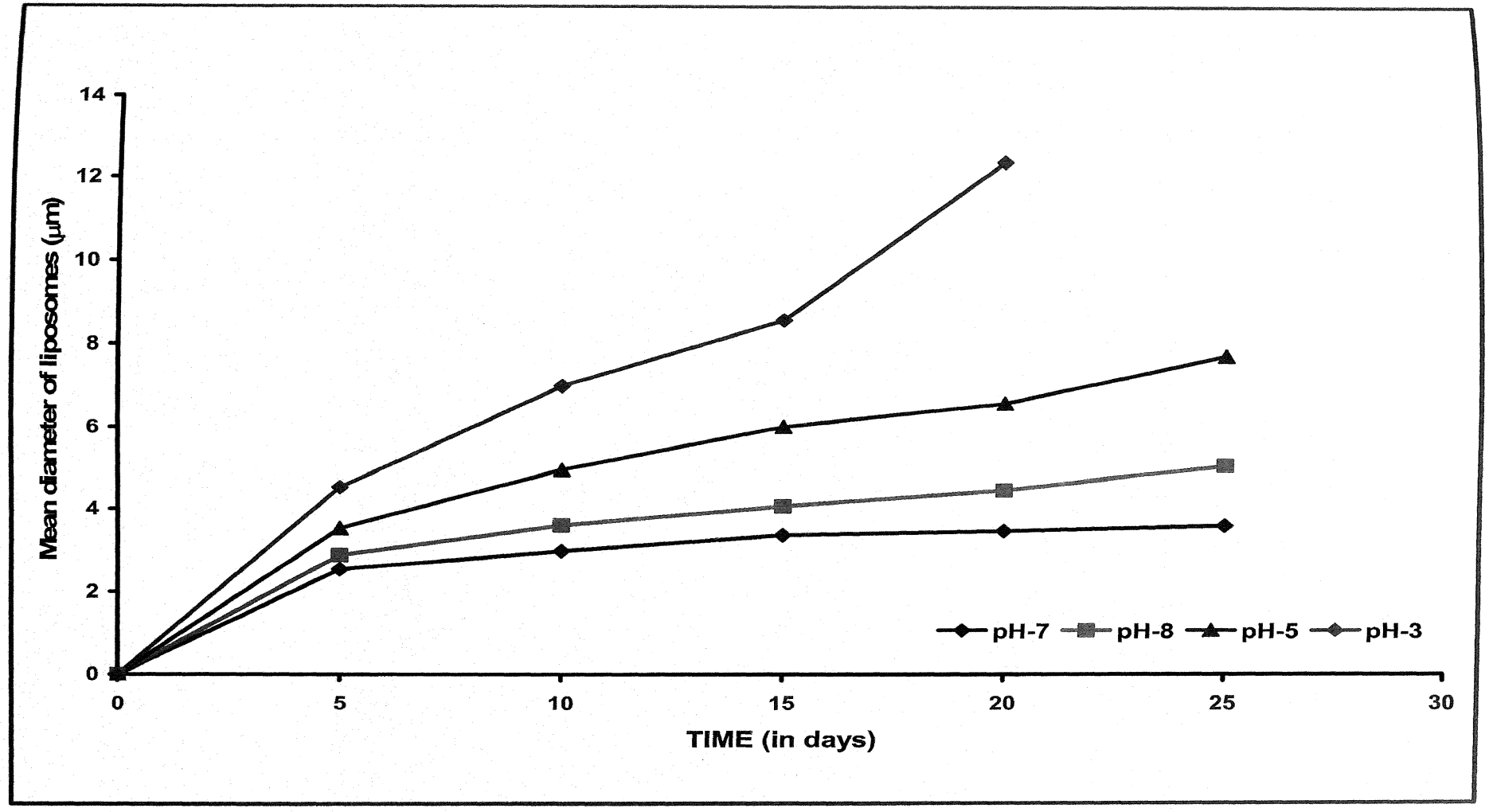


Figure-78: Effect of different pH on liposomes size enlargement

6.2.9 Effect of calcium chloride on liposomes size enlargement:

The conventional liposomes containing zidovudine were stored at different molar ratio of calcium chloride concentration. I.e. 0.001M, 0.01M, 0.1M solution at 25°C at pH7 reported. The influence of pH on liposomes diameter as a function of time have been investigated and are shown in fig-79 and values of the size enlargement of liposomes shown in table-47.in the range of 0.001M to 0.01M there is no significant change in the size enlargement. Above 0.01M i.e. 0.1M calcium chloride salt may induce aggregation behavior of conventional liposomes occurs only at a high concentration salt level.

Table-47 : Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement.

S. No	Time in days	0.001M CaCl2	0.01M $CaCl_2$	0.1M $CaCl_2$
0.	0	0	0	0
1.	5	2.48	2.88	4.13
2.	10	2.98	3.6	6.35
3.	15	3.37	4.07	7.05
4.	20	3.47	4.46	8.52
5.	25	3.6	5.06	10.45
6.	30	3.67	5.75	--

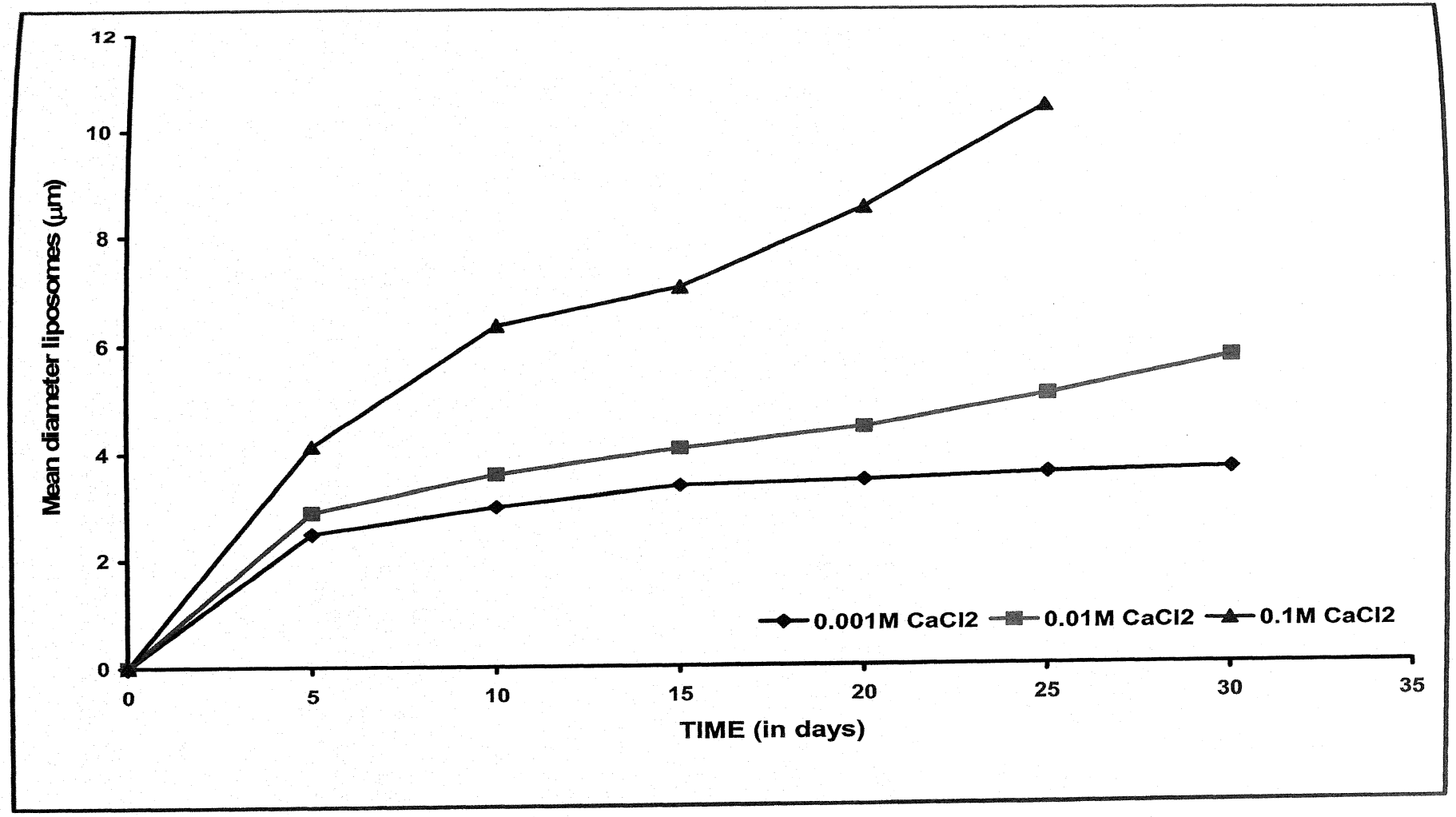


Figure-79: Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement

6.2.10 Stability studies of conventional liposome suspension:

The ability of vesicles to retain the drug (Drug retentive behavior) was assessed by keeping the liposomal suspension at different temperature conditions, i.e. 4°C, Room temperature, 37°C for a period of three months. Through out the study the liposomal suspensions were stored in aluminium foil sealed glass vials. The samples were taken every month and analyzed for the drug content spectrophotometricaly at 267 nm.

Table-48 : Stability of conventional liposomes containing zidovudine at 4°C after three months

S. No	Time in months	% drug retained
1.	Initial (zero month)	100
2.	1	99
3.	2	97.68
4.	3	95.72

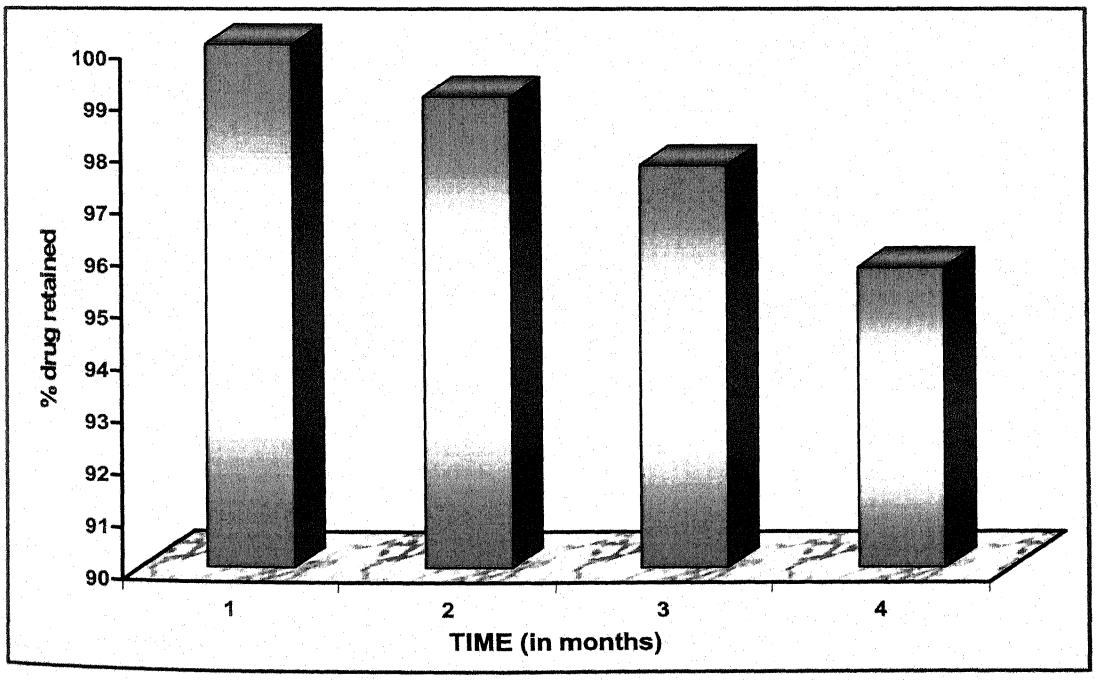


Figure-80: Stability of conventional liposomes containing zidovudine at **4°C** after three months

Figure-81: Stability of conventional liposomes containing zidovudine at **4°C** after three months

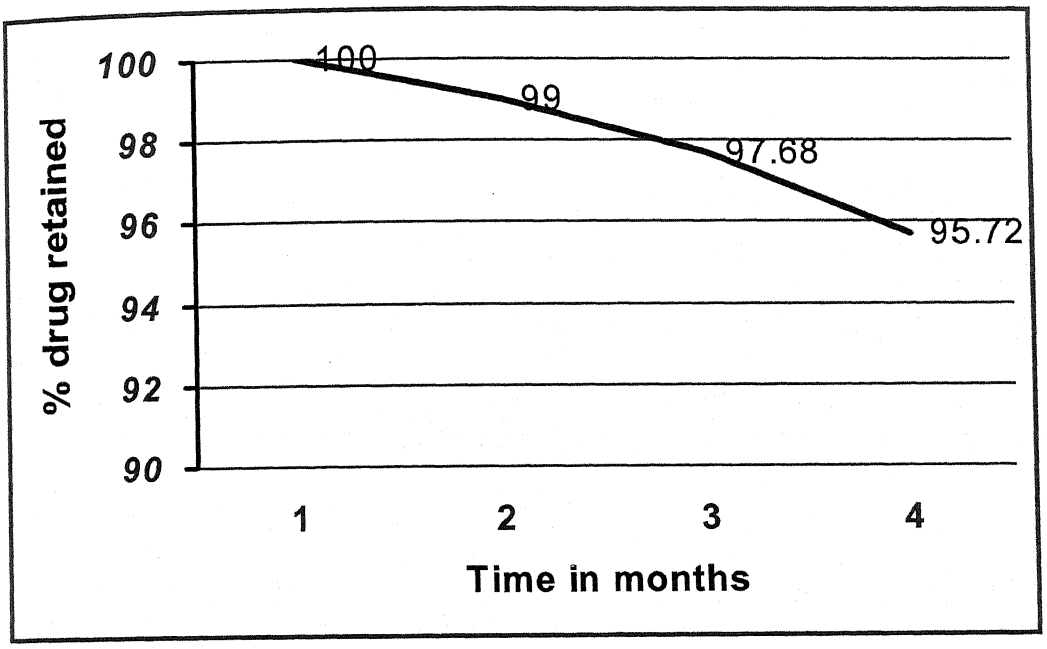


Table-49: Stability of conventional liposomes containing zidovudine at room temperature after three months.

S.No	Time in months	% drug retained
1.	Initial (zero month)	100
2.	1	76.12
3.	2	61.45
4.	3	49.75

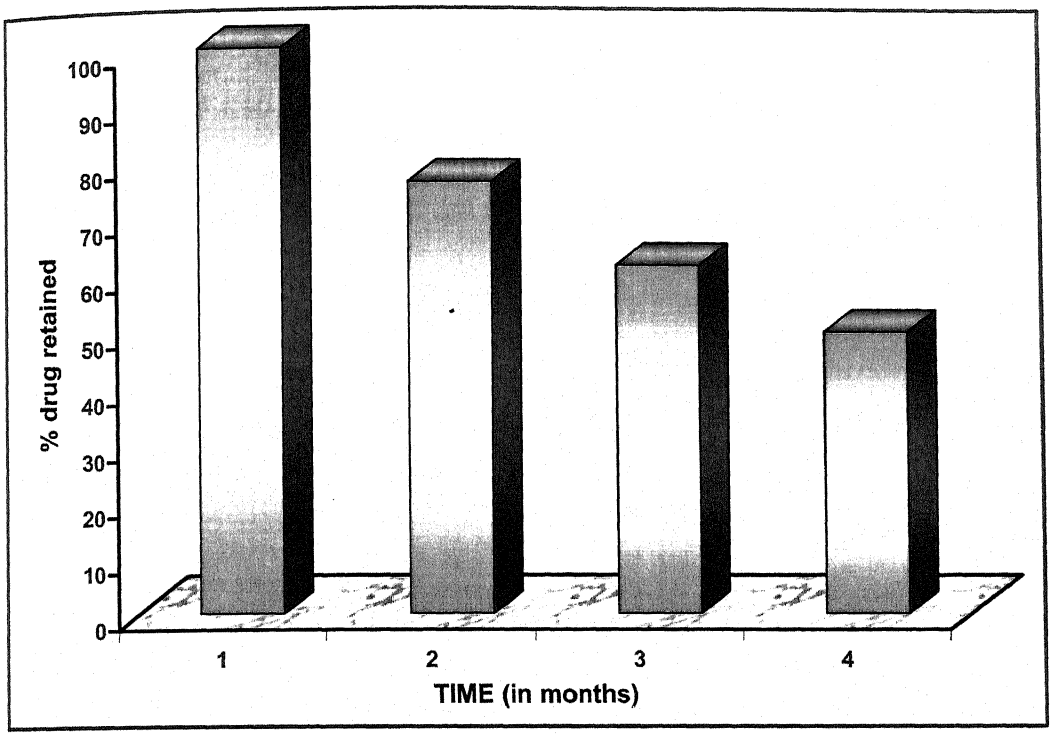


Figure-81(a) : Stability of conventional liposomes containing zidovudine at room temperature after three months.

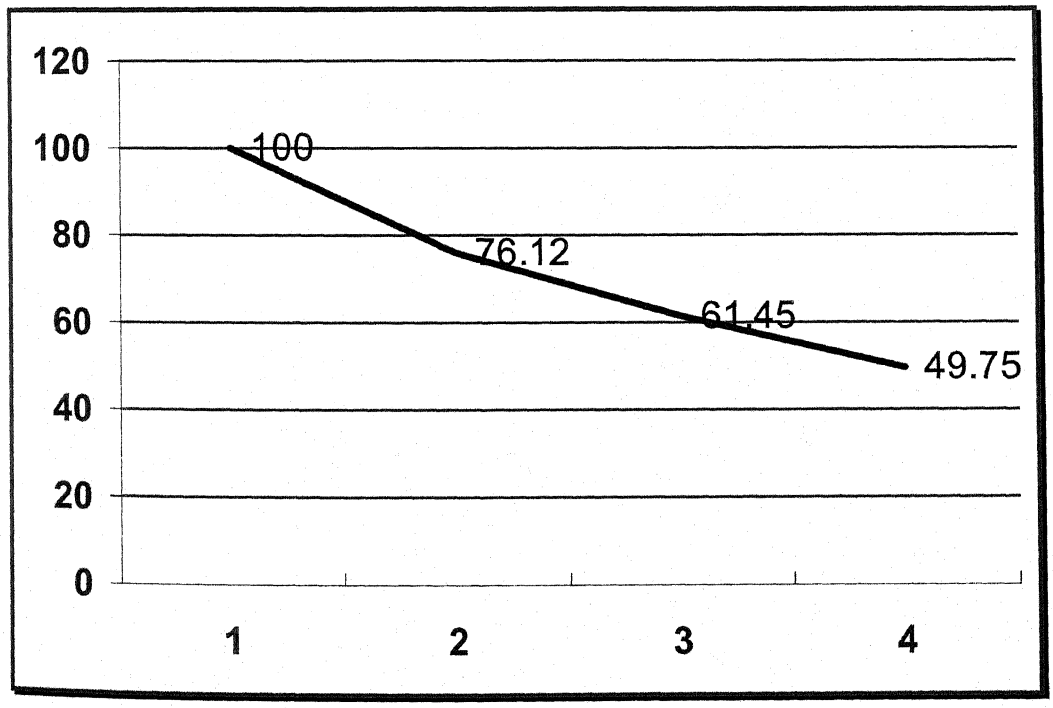


Figure-81(b) : Stability of conventional liposomes containing zidovudine at room temperature after three months.

Table-50: Stability of conventional liposomes containing zidovudine at 37°C after three months.

S.No	Time in months	% drug retained
1.	Initial (zero month)	100
2.	1	69
3.	2	41
4.	3	12

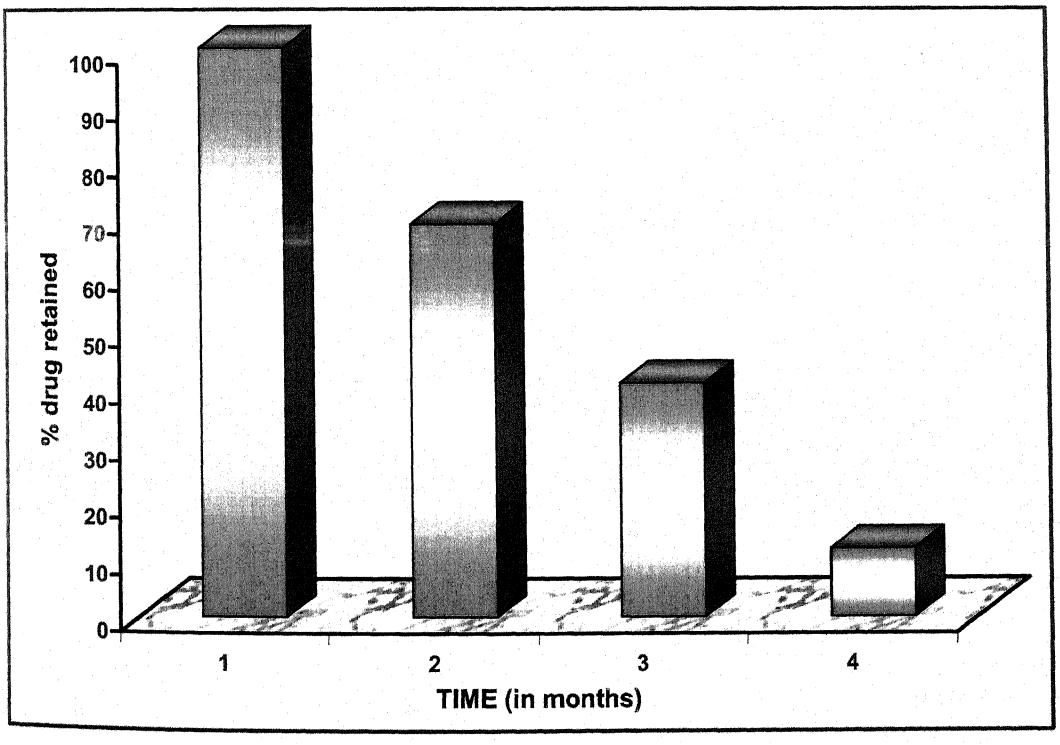


Figure-82: Stability of conventional liposomes containing zidovudine at room temperature, 37°C

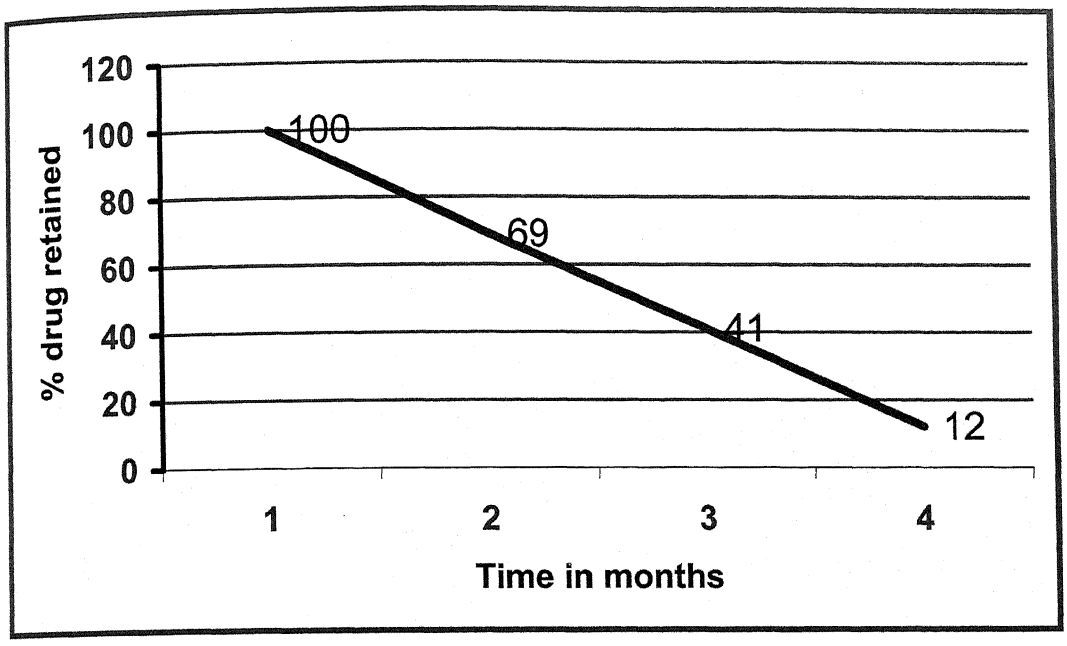


Fig-82(b) : Stability of conventional liposomes containing zidovudine at room temperature, 37°C

Table-51: Stability of conventional liposomes containing zidovudine at 4°C,roomtemprature, 37°C.

S.No	4°C	Room temperature	37°C
1.	100	100	100
2.	99	76.12	69
3.	97.68	61.45	41
4.	95.72	49.75	12

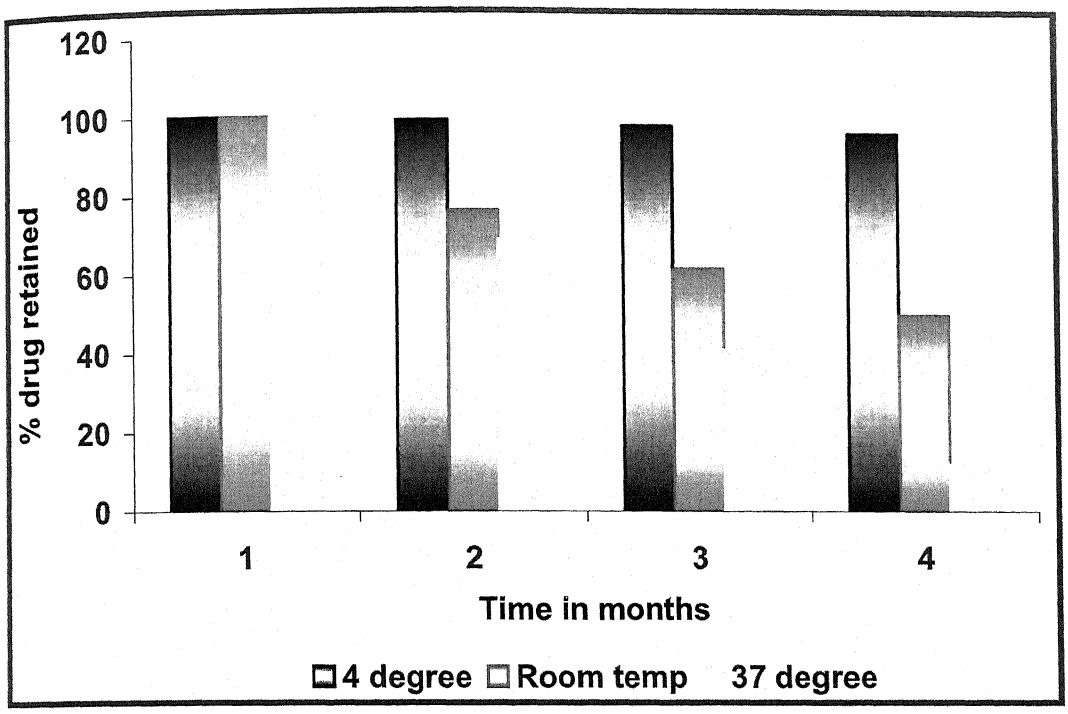


Figure-83(a) : Stability of conventional liposomes containing zidovudine at 4°C,roomtemprature, 37°C.

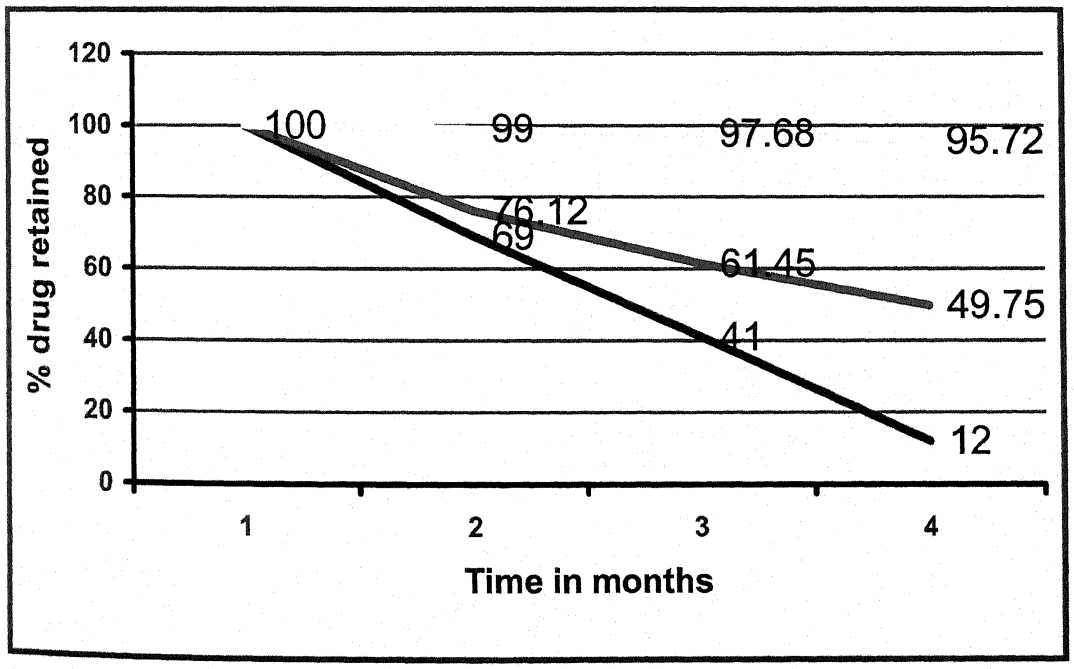


Figure-83(b) : Stability of conventional liposomes containing zidovudine at 4°C,roomtemprature, 37°C

Chapter- 7

Determination of Zeta Potential

Chapter-7

DETERMINATION OF ZETA POTENTIAL

7.1 Determination of Zeta Potential

The zeta potential is the overall charge a particle acquires in a particular medium. Both size and zeta potential can be measured on a Malvern Zetasizer.

7.2 Background of Liposomes

Liposomes are vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules, usually phospholipid. They can be prepared so that they entrap materials both within their aqueous compartment (water-soluble materials) and within the membrane (oil-soluble materials). They are extensively used as vehicles for the targeted delivery of drugs. The fate of intravenously injected liposomes is determined by a number of properties. The most important is zeta potential.

7.3 The Importance of Zeta Potential to Liposomes

The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential.

7.4 Uptake of Liposomes by the Body

A major problem in the use of liposomes for the delivery of drugs by oral or injection or topical routes into the blood stream is stability. Longer circulation times and controlled release can be achieved by coating the liposomes with a suitable polymer.

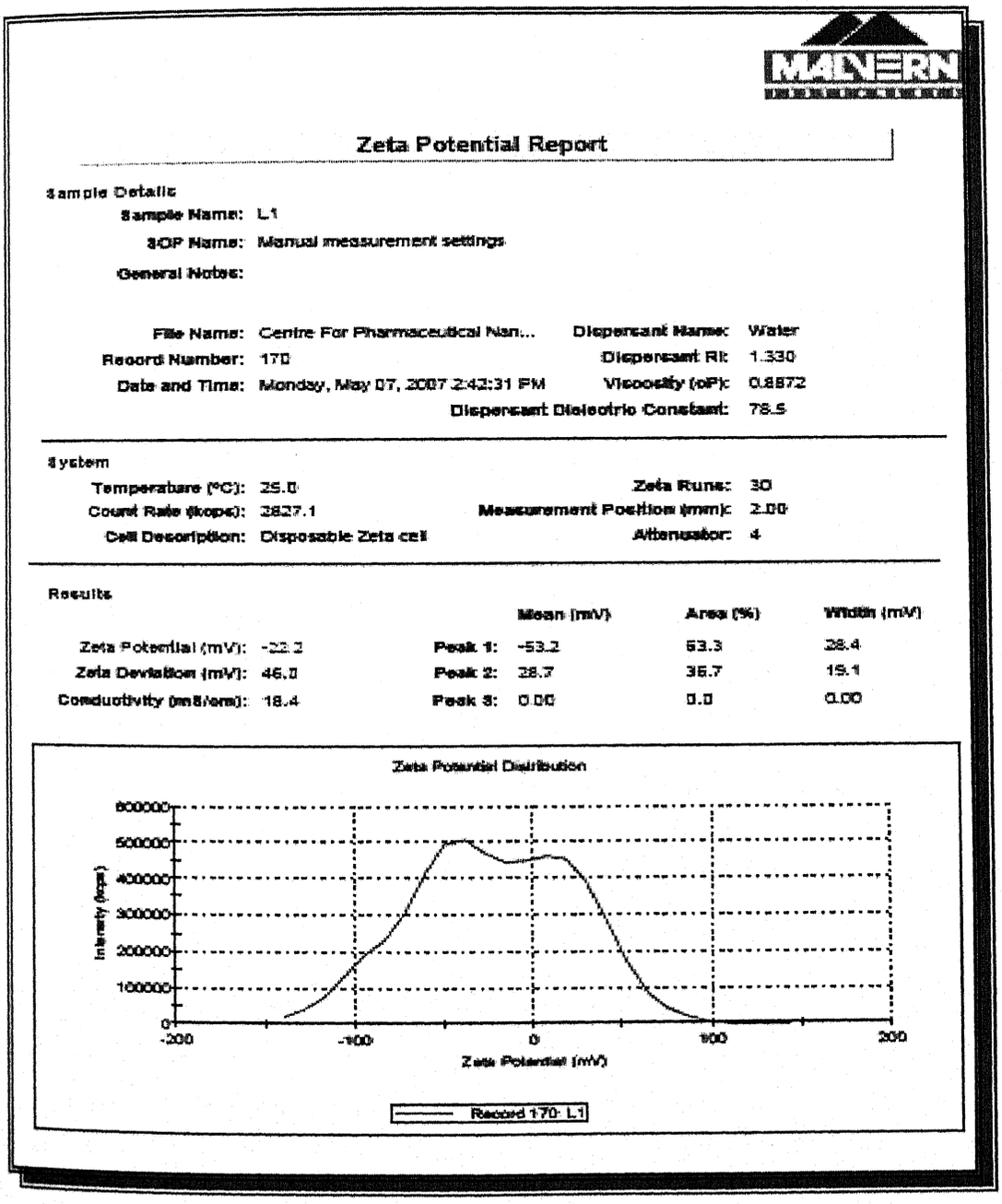
7.5 The Effect of Hydrophilic Polymers on Liposomes

The presence of hydrophilic polymers on the surface of the liposomes gives rise to a steric barrier and increases the stability of the liposomes. The most successful hydrophilic polymers to date are polyethylene glycols, HPMC, Carbopol, chitosan, polaxamer, and gelatin. Liposome with attached hydrophilic polymer on the surface.

7.6 Zeta Potential Study of the Effect of Cholesterol on Liposomes

The measured zeta potential of liposomes as a function of the concentration of Cholesterol and phospholipid (mole %). The zeta potential of 'naked' liposomes is –22.2mV.The zeta potential starts to decrease with decreasing concentration of cholesterol in to the phospholipid and eventually reaches a plateau around –5.91mV.

Figure-84: Zeta potential of liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 100mg



MALVERN

Zeta Potential Report

Sample Details

Sample Name: L1

SOP Name: Manual measurement settings

General Notes:

File Name: Centre For Pharmaceutical Nan...
Record Number: 170
Date and Time: Monday, May 07, 2007 2:42:31 PM
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 3827.1
Cell Description: Disposable Zeta cell
Zeta Runs: 30
Measurement Position (mm): 2.00
Attenuator: 4

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-22.2	Peak 1:	-53.2	53.3	28.4
Zeta Deviation (mV):	46.0	Peak 2:	28.7	36.7	15.1
Conductivity (mS/cm):	18.4	Peak 3:	0.00	0.0	0.00

Figure-85: Zeta size statistics of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 100mg

Size Statistics Report by Intensity

MALVERN INSTRUMENTS

Sample Details

Sample Name: L1

File Name: Centre For Pharmaceutical Nanotechnology.dts

SOP Name: Manual measurement settings

Measurement Date and Time: Friday, April 27, 2007 5:28:55 PM

Z-Average (nm):	683.0798	Derived Count Rate (kcps):	210615.144198...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.0		1110	2.6	
0.463	0.0		6.50	0.0		91.3	0.0		1280	0.5	
0.536	0.0		7.53	0.0		106	0.0		1480	0.0	
0.621	0.0		8.72	0.0		122	0.0		1720	0.0	
0.719	0.0		10.1	0.0		142	0.0		1990	0.0	
0.833	0.0		11.7	0.0		164	0.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.0		2670	0.0	
1.12	0.0		15.7	0.0		220	0.0		3090	0.0	
1.29	0.0		18.2	0.0		255	0.0		3580	0.0	
1.50	0.0		21.0	0.0		295	1.1		4150	0.6	
1.74	0.0		24.4	0.0		342	4.5		4800	2.0	
2.01	0.0		28.2	0.0		396	9.1		5560	3.9	
2.33	0.0		32.7	0.0		459	13.4		6440	0.0	
2.70	0.0		37.8	0.0		531	16.0		7460	0.0	
3.12	0.0		43.8	0.0		615	16.1		8630	0.0	
3.62	0.0		50.7	0.0		712	14.0		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	10.2				
4.85	0.0		68.1	0.0		955	6.0				

Statistics Graph (1 measurements)

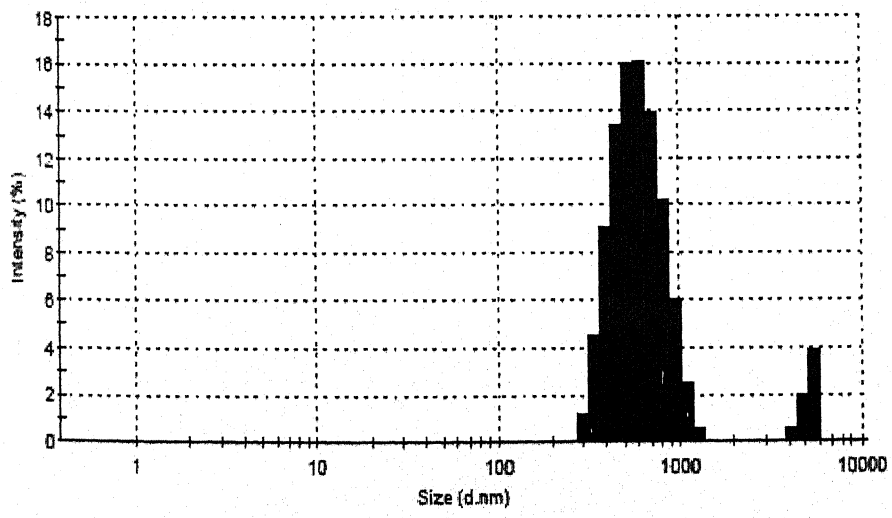


Figure-86: Size distribution of the liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 100mg

MALVERN INSTRUMENTS

Size Distribution Report by Intensity

Sample Details

Sample Name: L1

SOP Name: Manual measurement settings

General Notes:

File Name:	Centre For Pharmaceutica...	Dispersant Name:	Water
Record Number:	155	Dispersant RI:	1.330
Material RI:	1.50	Viscosity (cP):	0.8872
Material Absorbtion:	0.01	Measurement Date and Time:	Friday, April 27, 2007 5:28:55...

System

Temperature (°C):	25.0	Duration Used (s):	20
Count Rate (kcps):	186.1	Measurement Position (mm):	1.05
Cell Description:	Disposable sizing cuvette	Attenuator:	4

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	683	Peak 1:	617	93.5	192
PdI:	0.354	Peak 2:	5200	6.5	473
Intercept:	0.964	Peak 3:	0.00	0.0	0.00

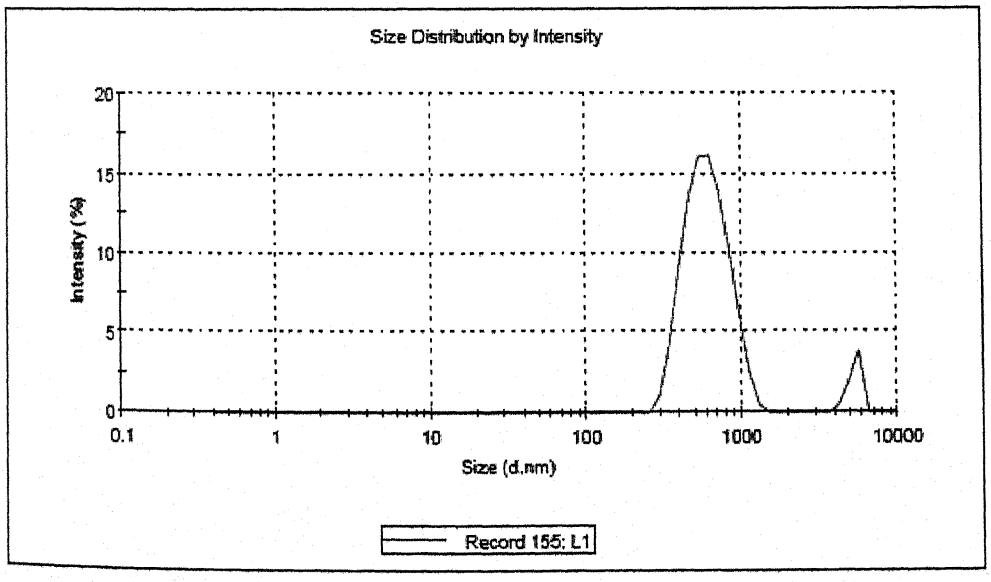


Figure-87: Zeta potential of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 80mg

MALVERN INSTRUMENTS

Zeta Potential Report

Sample Details

Sample Name: L2
SOP Name: Manual measurement settings
General Notes:

File Name:	Centre For Pharmaceutical Nan...	**Dispersant Name:**	Water
Record Number:	171	**Dispersant RI:**	1.330
Date and Time:	Monday, May 07, 2007 3:02:55 PM	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	30
Count Rate (kcps):	3182.2	**Measurement Position (mm):**	2.00
Cell Description:	Disposable Zeta cell	**Attenuator:**	5

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-21.2	**Peak 1:**	-21.2	100.0	24.2
Zeta Deviation (mV):	24.2	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	18.9	**Peak 3:**	0.00	0.0	0.00

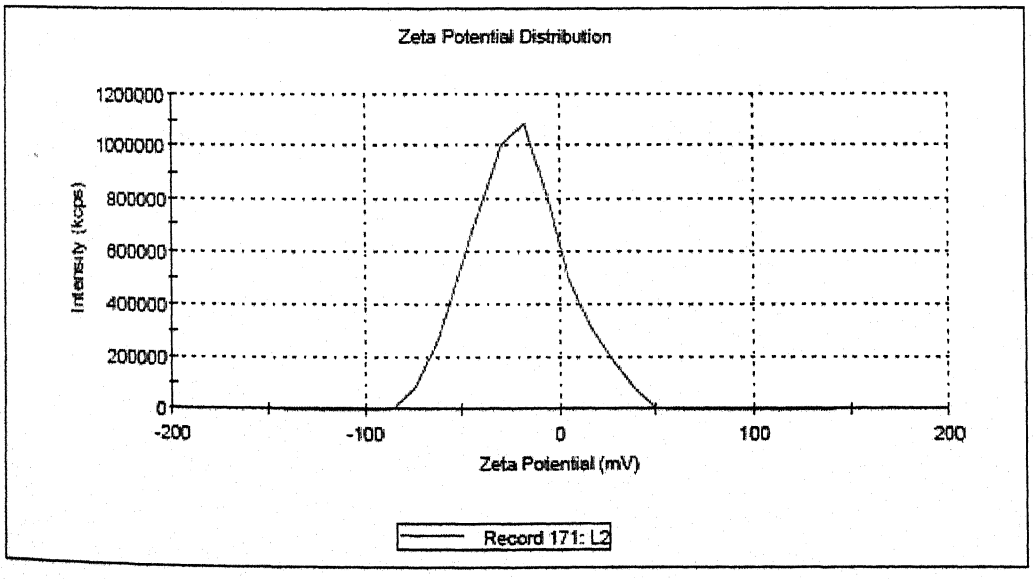


Figure-88: Zeta size statistics of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 80mg

Size Statistics Report by Intensity

MALVERN INSTRUMENTS

Sample Details

Sample Name: L2
File Name: Centre For Pharmaceutical Nanotechnology.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, April 27, 2007 5:24:49 PM

Z-Average (nm):	767.6181	Derived Count Rate (kcps):	366258.058386...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.0		1110	8.5	
0.463	0.0		6.50	0.0		91.3	0.0		1280	7.6	
0.536	0.0		7.53	0.0		106	0.0		1480	6.1	
0.621	0.0		8.72	0.0		122	0.1		1720	4.2	
0.719	0.0		10.1	0.0		142	0.4		1990	2.3	
0.833	0.0		11.7	0.0		164	1.1		2300	0.9	
0.965	0.0		13.5	0.0		190	1.9		2670	0.1	
1.12	0.0		15.7	0.0		220	2.6		3090	0.0	
1.29	0.0		18.2	0.0		255	3.3		3580	0.0	
1.50	0.0		21.0	0.0		295	3.7		4150	0.8	
1.74	0.0		24.4	0.0		342	4.0		4800	2.6	
2.01	0.0		28.2	0.0		396	4.3		5560	4.9	
2.33	0.0		32.7	0.0		459	4.8		6440	0.0	
2.70	0.0		37.8	0.0		531	5.4		7460	0.0	
3.12	0.0		43.8	0.0		615	6.4		8630	0.0	
3.62	0.0		50.7	0.0		712	7.4		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	8.2				
4.85	0.0		68.1	0.0		955	8.7				

Statistics Graph (1 measurements)

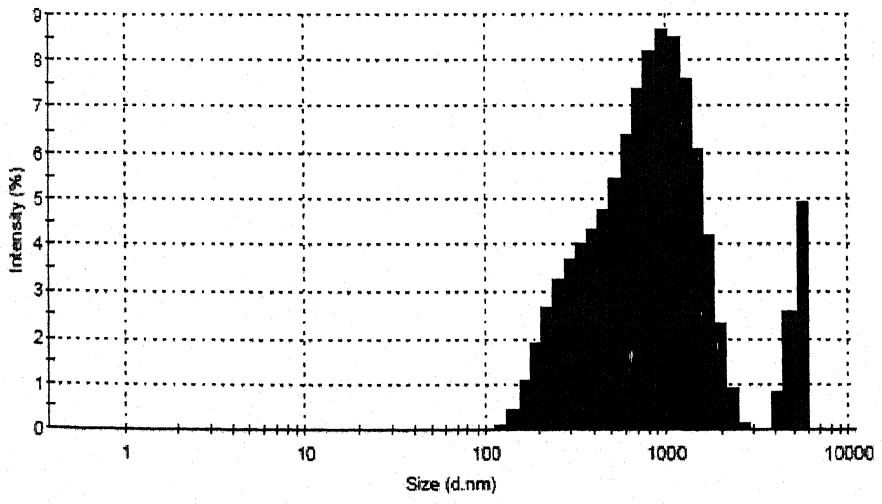


Figure-89: Size distribution of the liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 80mg

MALVERN INSTRUMENTS

Zeta Potential Report

Sample Details

Sample Name: L3

SOP Name: Manual measurement settings

General Notes:

File Name: Centre For Pharmaceutical Nan...
Record Number: 172
Date and Time: Monday, May 07, 2007 3:07:49 PM
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 3000.2
Cell Description: Disposable Zeta cell
Zeta Runs: 30
Measurement Position (mm): 2.00
Attenuator: 6

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-20.2	Peak 1:	-41.3	65.1	17.8
Zeta Deviation (mV):	29.3	Peak 2:	14.3	34.9	13.7
Conductivity (mS/cm):	17.9	Peak 3:	0.00	0.0	0.00

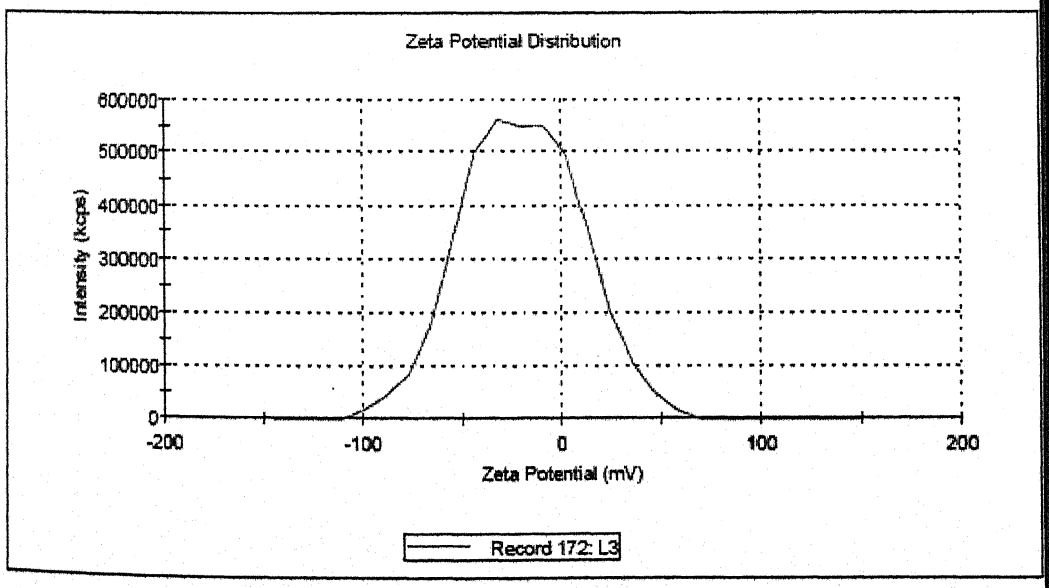


Figure-90 : Zeta potential of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 60mg

MALVERN

Size Distribution Report by Intensity

Sample Details

Sample Name: L2

SOP Name: Manual measurement settings

General Notes:

File Name:	Centre For Pharmaceutica...	Dispersant Name:	Water
Record Number:	154	Dispersant RI:	1.330
Material RI:	1.50	Viscosity (cP):	0.8872
Material Absorbtion:	0.01	Measurement Date and Time:	Friday, April 27, 2007 5:24:49...

System

Temperature (°C):	25.0	Duration Used (s):	20
Count Rate (kcps):	323.7	Measurement Position (mm):	0.85
Cell Description:	Disposable sizing cuvette	Attenuator:	4

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	768	Peak 1:	847	91.7	484
PdI:	0.544	Peak 2:	5190	8.3	482
Intercept:	0.791	Peak 3:	0.00	0.0	0.00

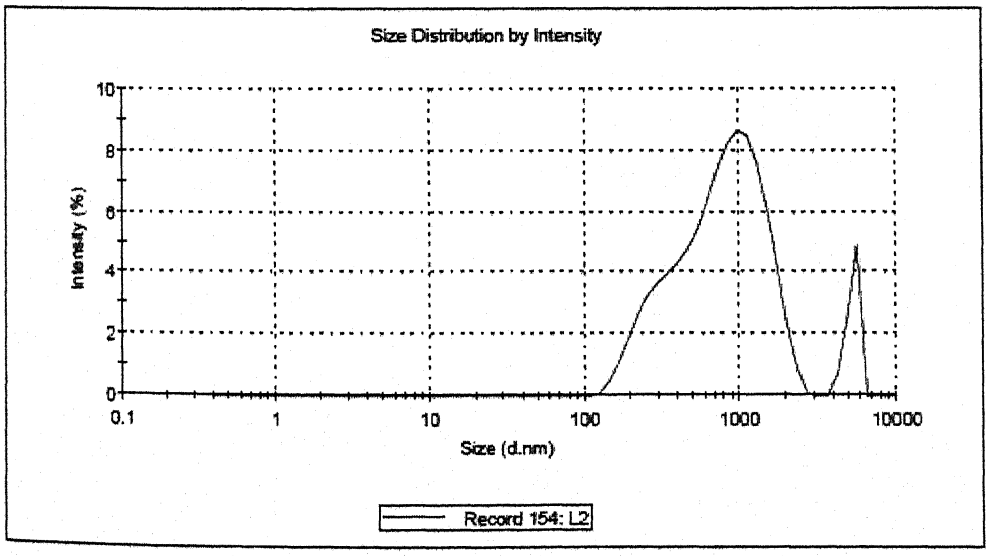


Figure-91: Zeta size statistics of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 60mg

Size Statistics Report by Intensity

MALVERN INSTRUMENTS

Sample Details

Sample Name: L3
File Name: Centre For Pharmaceutical Nanotechnology.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, April 27, 2007 5:19:48 PM

Z-Average (nm):	995.4107	Derived Count Rate (kcps):	301775.0124712
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.0		1110	0.8	
0.463	0.0		6.50	0.0		91.3	0.0		1280	0.0	
0.536	0.0		7.53	0.0		106	0.0		1480	0.0	
0.621	0.0		8.72	0.0		122	0.0		1720	0.1	
0.719	0.0		10.1	0.0		142	0.0		1990	0.9	
0.833	0.0		11.7	0.0		164	0.0		2300	2.5	
0.965	0.0		13.5	0.0		190	0.0		2670	4.5	
1.12	0.0		15.7	0.0		220	0.0		3090	6.5	
1.29	0.0		18.2	0.0		255	0.0		3580	8.0	
1.50	0.0		21.0	0.0		295	0.0		4150	8.6	
1.74	0.0		24.4	0.0		342	1.6		4800	8.2	
2.01	0.0		28.2	0.0		396	4.9		5560	7.0	
2.33	0.0		32.7	0.0		459	8.3		6440	0.0	
2.70	0.0		37.8	0.0		531	10.4		7460	0.0	
3.12	0.0		43.8	0.0		615	10.5		8630	0.0	
3.62	0.0		50.7	0.0		712	8.7		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	5.7				
4.85	0.0		68.1	0.0		955	2.8				

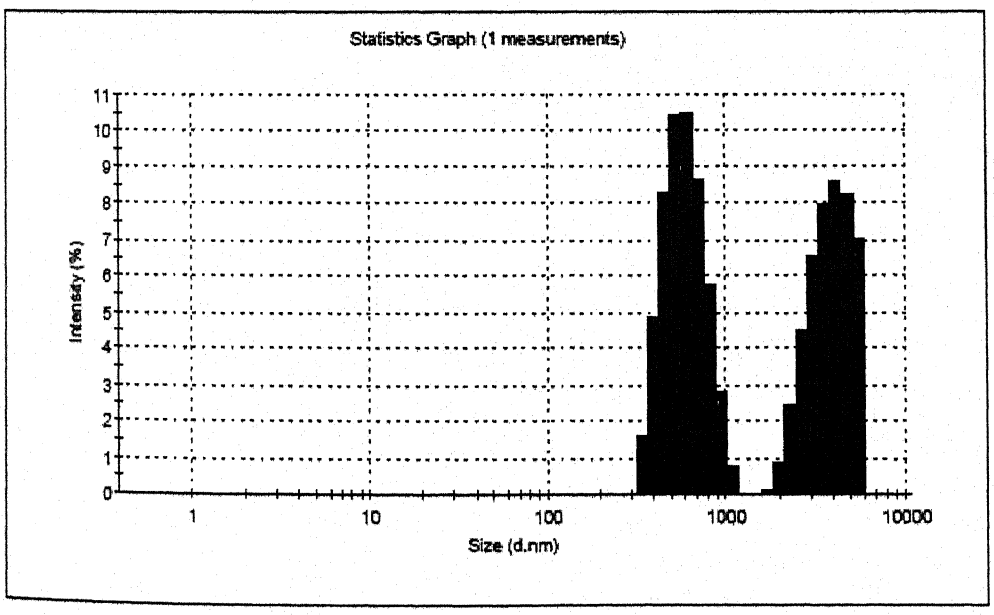


Figure-92: Size distribution of the liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 60mg

MALVERN INSTRUMENTS

Size Distribution Report by Intensity

Sample Details

Sample Name: L3

SOP Name: Manual measurement settings

General Notes:

File Name:	Centre For Pharmaceutica...	Dispersant Name:	Water
Record Number:	153	Dispersant RI:	1.330
Material RI:	1.50	Viscosity (cP):	0.8872
Material Absorbtion:	0.01	Measurement Date and Time:	Friday, April 27, 2007 5:19:48...

System

Temperature (°C):	25.0	Duration Used (s):	20
Count Rate (kcps):	266.7	Measurement Position (mm):	0.85
Cell Description:	Disposable sizing cuvette	Attenuator:	4

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	995	Peak 1:	610	53.7	166
PdI:	0.543	Peak 2:	3940	46.3	1020
Intercept:	0.887	Peak 3:	0.00	0.0	0.00

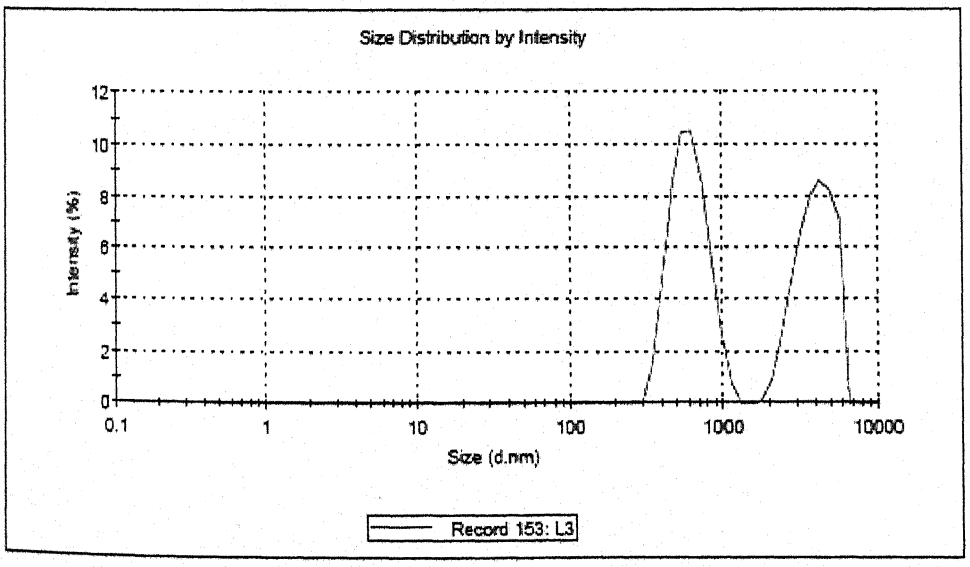


Figure-93: Zeta potential of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 40mg

MALVERN INSTRUMENTS

Zeta Potential Report

Sample Details

Sample Name: L4

SOP Name: Manual measurement settings

General Notes:

File Name:	Centre For Pharmaceutical Nan...	Dispersant Name:	Water
Record Number:	173	Dispersant RI:	1.330
Date and Time:	Monday, May 07, 2007 3:12:49 PM	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	30
Count Rate (kcps):	3134.1	Measurement Position (mm):	2.00
Cell Description:	Disposable Zeta cell	Attenuator:	7

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-19.6	Peak 1:	-19.6	100.0	19.7
Zeta Deviation (mV):	19.7	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	18.1	Peak 3:	0.00	0.0	0.00

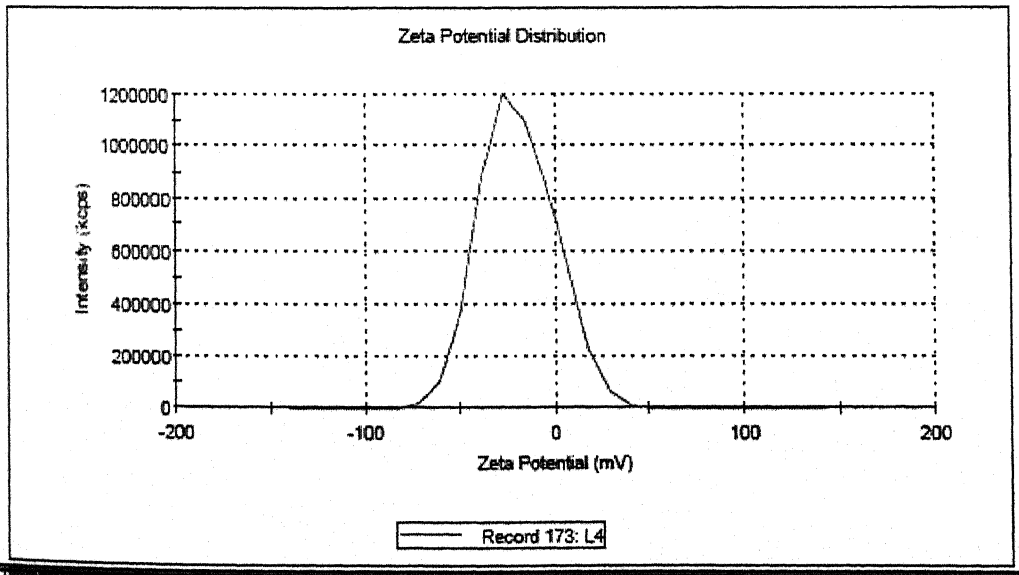


Figure-94: Zeta size statistics of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 40mg

Size Statistics Report by Intensity

Sample Details

Sample Name: L4
File Name: Centre For Pharmaceutical Nanotechnology.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Friday, April 27, 2007 5:33:09 PM

Z-Average (nm):	601.2173	Derived Count Rate (kcps):	225565.363907...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.3		1110	8.9	
0.463	0.0		6.50	0.0		91.3	1.1		1280	6.1	
0.536	0.0		7.53	0.0		106	1.8		1480	3.4	
0.621	0.0		8.72	0.0		122	2.0		1720	1.3	
0.719	0.0		10.1	0.0		142	1.7		1990	0.2	
0.833	0.0		11.7	0.0		164	1.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.3		2670	0.0	
1.12	0.0		15.7	0.0		220	0.0		3090	0.0	
1.29	0.0		18.2	0.0		255	0.0		3580	0.0	
1.50	0.0		21.0	0.0		295	0.3		4150	0.0	
1.74	0.0		24.4	0.0		342	1.6		4800	0.3	
2.01	0.0		28.2	0.0		396	3.8		5560	1.1	
2.33	0.0		32.7	0.0		459	6.6		6440	0.0	
2.70	0.0		37.8	0.0		531	9.4		7460	0.0	
3.12	0.0		43.8	0.0		615	11.7		8630	0.0	
3.62	0.0		50.7	0.0		712	12.9		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	12.8				
4.85	0.0		68.1	0.0		955	11.4				

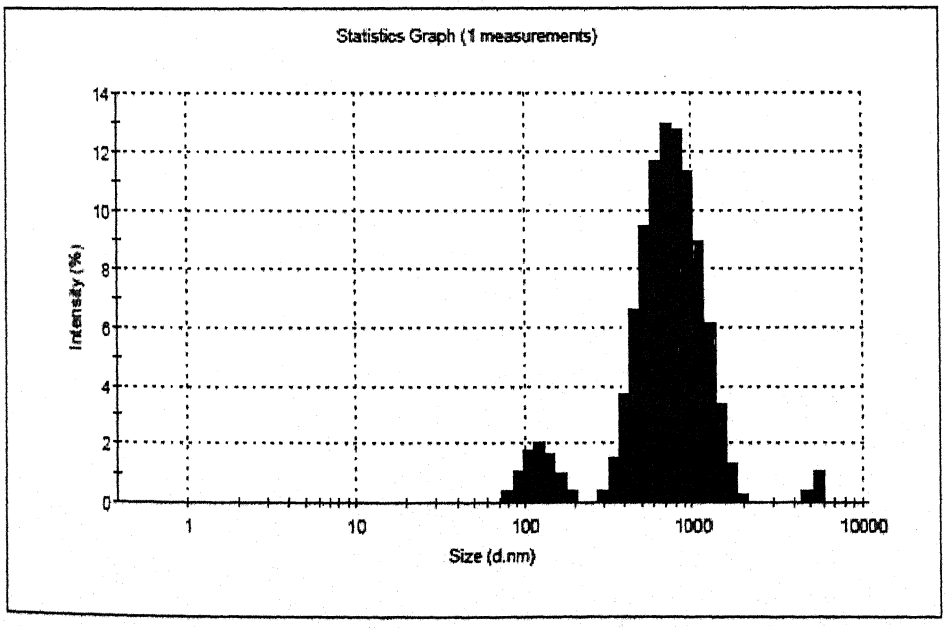


Figure-95 : Size distribution of the liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 40mg

MALVERN INSTRUMENTS

Size Distribution Report by Intensity

Sample Details

Sample Name: Sample C
SOP Name: Manual measurement settings
General Notes:

File Name:	NIPER.dts	Dispersant Name:	Water
Record Number:	82	Dispersant RI:	1.330
Material RI:	1.33	Viscosity (cP):	0.8872
Material Absorbtion:	0.00	Measurement Date and Time:	Monday, February 19, 2007 6...

System

Temperature (°C):	25.0	Duration Used (s):	10
Count Rate (kcps):	167.0	Measurement Position (mm):	0.65
Cell Description:	Disposable sizing cuvette	Attenuator:	3

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	2040	Peak 1:	247	88.8	29.2
PdI:	1.000	Peak 2:	38.8	11.2	3.64
Intercept:	0.859	Peak 3:	0.00	0.0	0.00

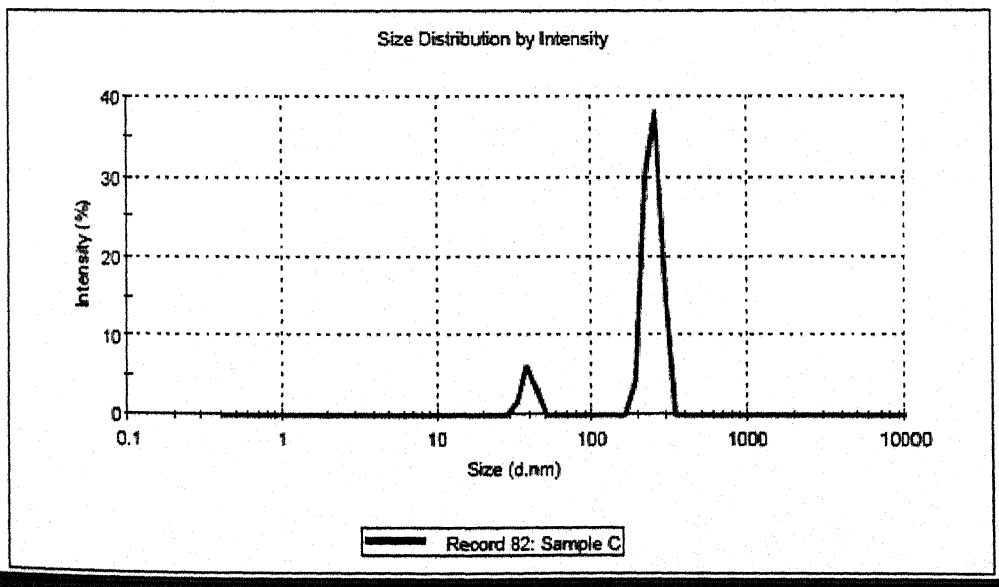


Figure-96 : Zeta potential of liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 30mg

MALVERN INSTRUMENTS

Zeta Potential Report

Sample Details

Sample Name: Sample B

SOP Name: Manual measurement settings

General Notes:

File Name:	NIPER.dts	Dispersant Name:	Water
Record Number:	86	Dispersant RI:	1.330
Date and Time:	Monday, February 19, 2007 7:19:...	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	20
Count Rate (kcps):	1795.0	Measurement Position (mm):	2.00
Cell Description:	Disposable Zeta cell	Attenuator:	10

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-7.99	Peak 1:	-7.99	100.0	14.3
Zeta Deviation (mV):	14.3	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	1.25	Peak 3:	0.00	0.0	0.00

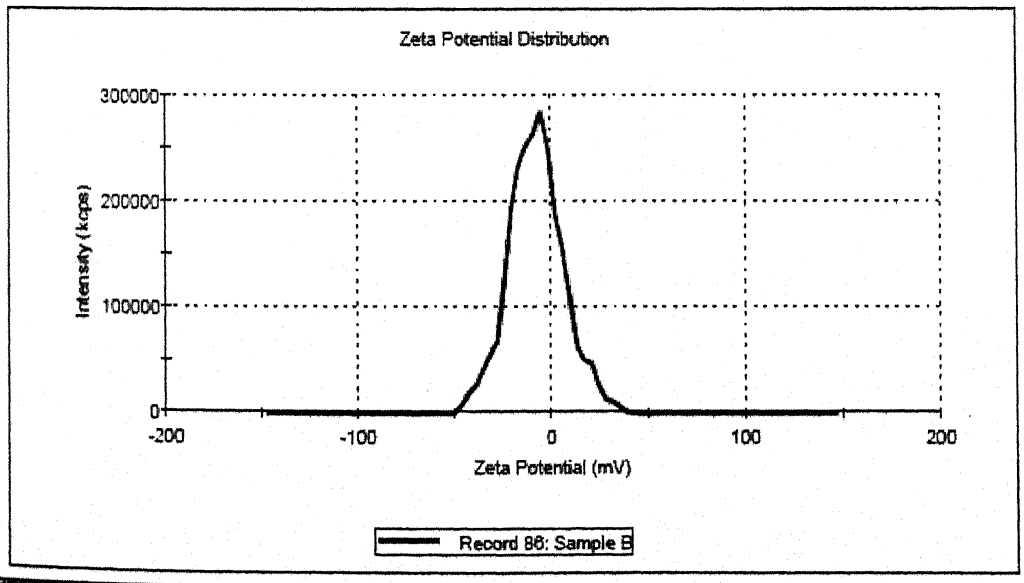


Figure-97 : Zeta size statistics of liposomes containing phosphatidyl choline and cholesterol in the ratio of 200mg: 30mg

Size Statistics Report by Intensity

MALVERN INSTRUMENTS

Sample Details

Sample Name: Sample B
File Name: NIPER.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Monday, February 19, 2007 6:53:36 PM

Z-Average (nm):	1307.814	Derived Count Rate (kcps):	614285.202771...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.402	0.0		5.61	0.0		78.8	0.0		1110	0.0	
0.463	0.0		6.50	0.0		91.3	0.0		1280	0.0	
0.535	0.0		7.53	0.0		106	0.0		1480	0.0	
0.621	0.0		8.72	0.0		122	0.0		1720	0.0	
0.719	0.0		10.1	0.0		142	0.0		1990	0.0	
0.833	0.0		11.7	0.0		164	0.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.0		2670	0.0	
1.12	0.0		15.7	0.0		220	10.4		3090	0.0	
1.29	0.0		18.2	0.0		255	31.1		3580	0.0	
1.50	0.0		21.0	0.0		295	30.5		4150	0.0	
1.74	0.0		24.4	0.0		342	8.0		4800	0.0	
2.01	0.0		28.2	0.0		396	0.0		5560	0.0	
2.33	0.0		32.7	5.0		459	0.0		6440	0.0	
2.70	0.0		37.8	9.3		531	0.0		7460	0.0	
3.12	0.0		43.8	5.6		615	0.0		8630	0.0	
3.62	0.0		50.7	0.0		712	0.0		1.00e4	0.0	
4.19	0.0		58.6	0.0		825	0.0				
4.85	0.0		68.1	0.0		955	0.0				

Statistics Graph (1 measurements)

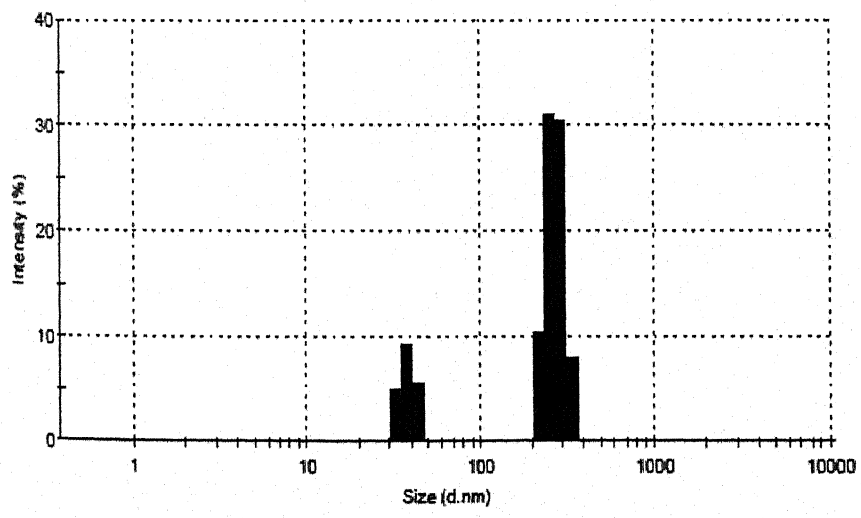


Figure-98 : Size distribution of the liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 30mg

MALVERN INSTRUMENTS

Size Distribution Report by Intensity

Sample Details

Sample Name: Sample B
SOP Name: Manual measurement settings
General Notes:

File Name:	NIPER.dls	Dispersant Name:	Water
Record Number:	81	Dispersant RI:	1.330
Material RI:	1.33	Viscosity (cP):	0.8872
Material Absorbtion:	0.00	Measurement Date and Time:	Monday, February 19, 2007 6...

System

Temperature (°C):	25.0	Duration Used (s):	10
Count Rate (kcps):	156.2	Measurement Position (mm):	0.85
Cell Description:	Disposable sizing cuvette	Attenuator:	3

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	1310	Peak 1:	275	80.1	34.0
PdI:	0.970	Peak 2:	38.2	19.9	4.08
Intercept:	0.935	Peak 3:	0.00	0.0	0.00

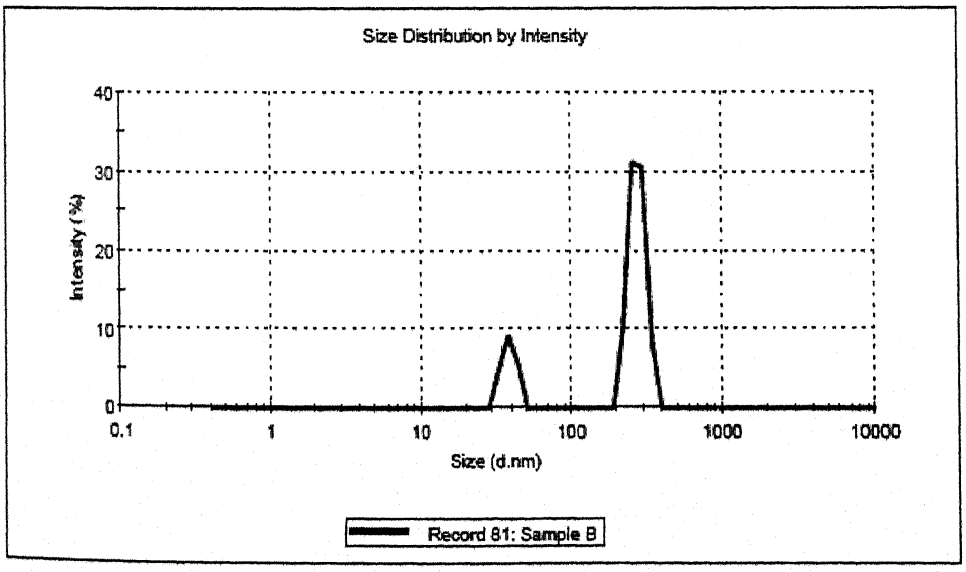


Figure-99: Zeta potential of liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 20mg

MALVERN INSTRUMENTS

Zeta Potential Report

Sample Details

Sample Name: Sample A

SOP Name: Manual measurement settings

General Notes:

File Name:	NIPER.dts	Dispersant Name:	Water
Record Number:	87	Dispersant RI:	1.330
Date and Time:	Monday, February 19, 2007 7:24:...	Viscosity (cP):	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	Zeta Runs:	20
Count Rate (kcps):	1748.3	Measurement Position (mm):	2.00
Cell Description:	Disposable Zeta cell	Attenuator:	10

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-5.91	Peak 1:	-5.91	100.0	34.5
Zeta Deviation (mV):	34.5	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	8.47	Peak 3:	0.00	0.0	0.00

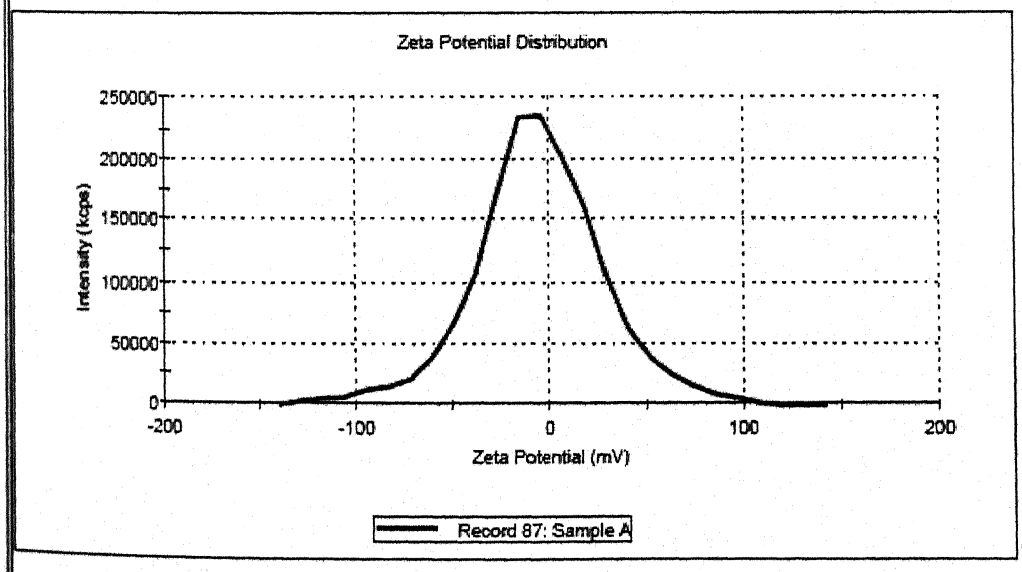


Figure-100 : Zeta size statistics of liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 20mg

Size Statistics Report by Intensity

MALVERN INSTRUMENTS

Sample Details

Sample Name: Sample A
File Name: NIPER.dts
SOP Name: Manual measurement settings
Measurement Date and Time: Monday, February 19, 2007 4:46:16 PM

Z-Average (nm):	1103.509	Derived Count Rate (kcps):	2607220.25366...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %	Size d.nm	Mean Intensity %	Std Dev Intensity %
0.400	0.0		5.61	0.0		78.8	0.2		1110	21.7	
0.463	0.0		6.50	0.0		91.3	3.3		1280	15.7	
0.536	0.0		7.53	0.0		106	5.6		1480	6.6	
0.621	0.0		8.72	0.0		122	4.8		1720	0.3	
0.719	0.0		10.1	0.0		142	2.0		1990	0.0	
0.833	0.0		11.7	0.0		164	0.0		2300	0.0	
0.965	0.0		13.5	0.0		190	0.0		2670	0.0	
1.12	0.0		15.7	0.0		220	0.0		3090	0.0	
1.29	0.0		18.2	0.0		255	0.0		3580	0.0	
1.50	0.0		21.0	0.0		295	0.0		4150	0.0	
1.74	0.0		24.4	0.0		342	0.0		4800	0.0	
2.01	0.0		28.2	0.0		396	0.0		5560	0.0	
2.33	0.0		32.7	0.0		459	0.0		6440	0.0	
2.70	0.0		37.8	0.0		531	0.0		7460	0.0	
3.12	0.0		43.8	0.0		615	0.0		8630	0.0	
3.62	0.0		50.7	0.0		712	5.1		1.00e4	0.0	
4.19	0.0		58.8	0.0		825	13.9				
4.85	0.0		68.1	0.0		955	20.9				

Statistics Graph (1 measurements)

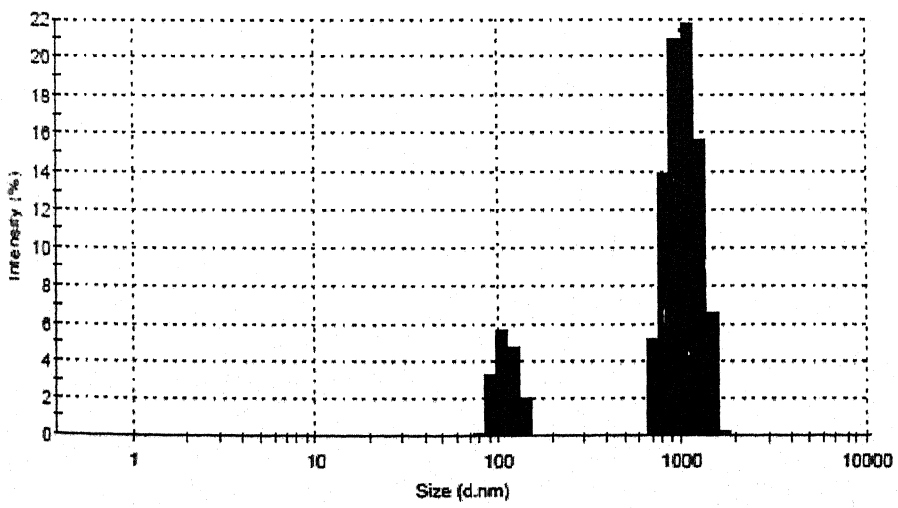


Figure-101: Size distribution of the liposomes containing Phosphotidyl choline and cholesterol in the ratio of 200mg: 20mg

MALVERN INSTRUMENTS

Size Distribution Report by Intensity

Sample Details

Sample Name: Sample A

SOP Name: Manual measurement settings

General Notes:

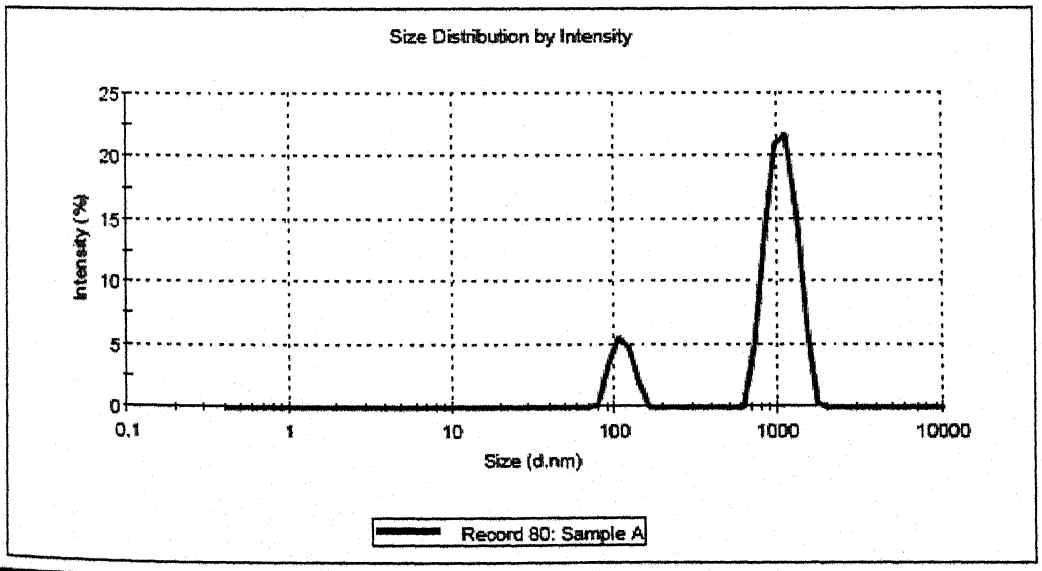
File Name:	NIPER.dts	Dispersant Name:	Water
Record Number:	80	Dispersant RI:	1.330
Material RI:	1.33	Viscosity (cP):	0.8872
Material Absorbtion:	0.00	Measurement Date and Time:	Monday, February 19, 2007 4..

System

Temperature (°C):	25.0	Duration Used (s):	10
Count Rate (kcps):	86.0	Measurement Position (mm):	0.85
Cell Description:	Disposable sizing cuvette	Attenuator:	2

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	1100	Peak 1:	1060	84.2	210
PdI:	0.887	Peak 2:	112	15.8	16.0
Intercept:	0.972	Peak 3:	0.00	0.0	0.00



A GENERAL INTRODUCTION TO GEL

A gel is a soft, solid or solid-like material consisting of two or more components, one of which is a liquid, present in substantial quantity. A gel should, on a time scale of seconds, not flow under the influence of its own weight. The term "hydrogel" was introduced by Thomas Graham in 1864 to denote silicic acid hydrates with gelatinous properties[124]. The first biological uses of gels (polymerized methylmethacrylate) were presented by the Institute for Macromolecular Chemistry in Prague in 1960. Gelation occurs via the cross-linking of polymer chains, something that can be achieved by (i) covalent bond formation (chemical cross-linking) or (ii) non-covalent bond formation (physical cross-linking). Examples of non-covalent bonds are hydrogen bonds and the cross-linking of polyelectrolyte by inorganic ions[125]. Gels have been used for the delivery of drugs for both systemic and local action[126]. Many different methods using gels have been reported, including subcutaneous delivery for sustained release[127], buccal delivery[128], delivery to the stomach[129], colon[130], rectum[131] and vagina[132]. Gel formulations with suitable rheological properties increase the contact time with the mucosa at the site of absorption. The increased contact time is caused by the mucoadhesive properties of the polymer in the gel and by the rheological properties of the formulation reducing the clearance by the skin. The rheological and mucoadhesive properties of a gel may give rise to a long residence time, but this is only advantageous if the drug remains in the formulation and is released throughout the complete period. The viscosity of a gel may increase dramatically in the presence of a polymer, but the transport conditions for a large drug molecule are difficult. There are several ways to sustain the release of a drug from gels in order to take full advantage in contact time. The drug can be dispersed in the gel giving a release and target concentration that is higher than that corresponding to the solubility of drug, formulated as particles, distributed in liposomes, that gives zero order release.

Picture showing Franz Diffusion Cell

Chapter-8

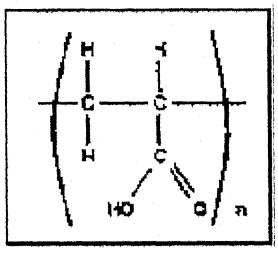
Carbopol

CARBOPOL

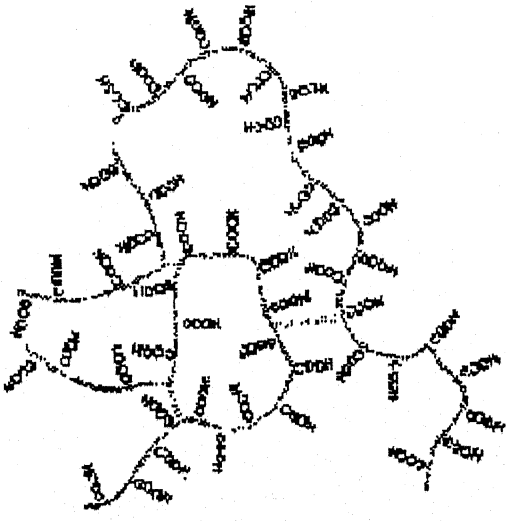
Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are produced from primary polymer particles of about 0.2 to 6.0 micron average diameter. The flocculated agglomerates cannot be broken into the ultimate particles when produced. Each particle can be viewed as a network structure of polymer chains interconnected via cross-linking[133].

The objective of this study was to develop a suitable method for the recovery of a hydrophilic drug in liposomes from controlled-release gel containing carbopol. To develop a method to recover a hydrophilic drug from controlled-release gels containing carbopol, Zidovudine-was chosen as the model drug. It has a rapid onset of action coupled with a relatively short half-life, and is indicated for managing HIV. The drug substance occurs as a white to off-white crystalline powder.

8.1 GENERAL STRUCTURE OF CARBOPOL



General Structure of Carbopol Polymers



Schematic drawing of a molecular segment of a cross-linked polyacrylic acid polymer.

Carbomers readily absorb water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and its essentially insolubility in water makes Carbopol a potential candidate for use in controlled release drug delivery system[134]. Carbopol polymers are offered as fluffy, white, dry powders (100% effective). The carboxyl groups provided by the acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol polymers have an average equivalent weight of 76 per carboxyl group[135]. Carbopol 71G, 971 P, 974 P are cross-linked with allyl penta erythritol and polymerized in ethyl acetate. Polycarbophil is cross-linked polymer in divinyl glycol and polymerized in solvent benzene. All the polymers fabricated in ethyl acetate are neutralized by 1-3% potassium hydroxide. Though Carbopol 971 P and Carbopol 974 P are manufactured by same process under similar conditions, the difference in them is that Carbopol 971 P has slightly lower level of cross-linking agent than Carbopol 974 P. Carbopol 71 G is the granular form Carbopol grade. Depending upon the degree of cross-linking and manufacturing conditions, various grades of Carbopol are available. Each grade is having its significance for its usefulness in pharmaceutical dosage forms [136].

The three dimensional nature of these polymers confers some unique characteristics, such as biological inertness, not found in similar linear polymers. The Carbopol resins are hydrophilic substances that are not soluble in water. Rather, these polymers swell when dispersed in water forming colloidal, mucilage-like dispersion.Carbopol polymers are bearing very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Because the pKa of these polymers is 6.0 to 0.5, the carboxylate moiety on the polymer backbone ionizes, resulting in repulsion between the native charges, which adds to the swelling of the polymer. The glass transition temperature of Carbopol polymers is 105°C (221°F) in powder form. However, glass transition temperature decreases significantly as the polymer comes into contact of water. The polymer chains start gyrating and radius of gyration becomes increasingly larger. Macroscopically, this phenomenon manifests itself as swelling[137].

8.2 PROPERTIES OF CARBOPOL:

Appearance	:	Fluffy, white, mildly acidic polymer
Bulk Density	:	Approximately 208 kg/m^3
Specific gravity	:	1.41
Moisture content	:	2.0% maximum
Equilibrium moisture content	:	8-10% (at 50% relative humidity)
PKa	:	6.0 ± 0.5
pH of 1.0% water dispersion	:	2.5 - 3.0
pH of 0.5% water dispersion	:	2.7 - 3.5
Ash content	:	0.009 ppm (average)

The molecular weights between adjacent crosslinks (Mc) are approximately inversely proportional to the crosslinker density. These may be calculated from the functionality of the crosslinking monomer, the relative ratio of acrylic acid to crosslinking monomer, and the efficiency of the crosslinking reaction, assuming negligible chain ends. Based on this approach, Taylor calculated Mc for Carbopol 941 in the order of several million. This number is far too high as compared to the theoretical Mc calculated from the stoichiometry. Carnali and Naser estimated the Mc for Carbopol 941 to be 3,300 monomer units (or 3,300 x 72 = 237,600 gm/mole) derived from a combination of dilute solution viscosity and equilibrium swelling. The Mc reported for Carbopol 940 was 1,450 monomer units (or 1,450 x 72 = 104,400 gm/mole).

Alternatively, the molecular weight can be qualitatively compared to the rheological properties of a swollen gel and/or from the equilibrium-swelling ratio. In simple terms, low viscosity, low rigidity polymers, such as Carbopol 941 and Carbopol 971P, have a higher Mc. Conversely, they have lower crosslinker densities. The higher the crosslinker level, the lower the equilibrium swelling ratio. In the network theory of elasticity, the elastic modulus, G, is inversely proportional to the molecular weight between crosslink's (Mc). There have been attempts to extend the elasticity theory to swollen gels.

Viscosity range of different Carbopol Polymers

1.	Cbl 934 NF-30500 – 39400	7	Cbl 5984 EP-30500 – 39400
2.	Cbl 940 NF-40000 – 60000	8	Cbl 934 P NF-29400 – 39400
3.	Cbl 941 NF-4000 – 10000	9	Cbl 971 P NF-4000 – 11000
4.	Cbl 980 NF-40000 – 60000	10	Cbl 974 P NF-29400 – 39400
5.	Cbl 981 NF- 4000 – 10000	11	Cbl 71 G NF-4000 – 11000
6.	Cbl 1342 NF-9500 – 26500		

Cbl – Carbapol*

8.3 APPLICATIONS:

- Controlled release in tablets[138].
- Bioadhesion in buccal [139], intestinal [140], vaginal [141] and rectal[142] applications.
- Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs[143].
- Permanent suspensions of insoluble ingredients in oral suspensions and topicals[144].
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants.

8.3.1 Bioadhesive Applications[145]:

Bioadhesion is a surface phenomena in which a material may be of natural or synthetic origin, adheres or stick to biological surface, usually mucus membrane. The concept of bioadhesion is emerging as a potential application in drug delivery due to its applicability for bioavailability enhancement, prolongation of residence time for drug in GIT and better contact between drug and absorbing surface.

Many hydrophilic polymers adhere to mucosal surfaces as they attract water from the mucus gel layer adherent to the epithelial surface. This is the simplest mechanism of adhesion and has been defined as "adhesion by hydration" Various kinds of adhesive force, e.g. hydrogen bonding between the adherent polymer and the substrate, i.e. mucus, are involved in mucoadhesion at the molecular level. Carbopol polymers have been demonstrated to create a tenacious bond with the

mucus membrane resulting in strong bioadhesion. Many commercial oral and topical products available today and under investigation have been formulated with Carbopol polymers, as they provide numerous benefits in bioadhesive formulations.

8.3.2 Benefits in Bioadhesive Applications –

- Improve bioavailability of certain drugs.
- Enhance patient compliance (fewer doses are needed per day)
- Lower concentrations of the active ingredients can be used.
- Provide excellent adhesion forces.

8.3.3 Topical Applications[146,147] :

Carbomers are very well suited to aqueous formulations of the topical dosage forms. Many commercial topical products available today have been formulated with these polymers, as they provide the following numerous benefits to topical formulations:

- Safe & Effective — Carbopol polymers have a long history of safe and effective use in topical gels, creams, lotions, and ointments. They are also supported by extensive toxicology studies.
- Non-Sensitizing — Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage.
- No Effect on the Biological Activity of the Drug — Carbopol polymers provide an excellent vehicle for drug delivery. Due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug.
- Excellent Thickening, Suspending, & Emulsification Properties for Topical Formulations

Products with a wide range of viscosities and flow properties have been successfully formulated and commercialized. Carbopol polymers are used to permanently suspend the active ingredients in transdermal reservoirs as well as in topical gels and creams. Pemulen polymeric emulsifiers can be used to prepare stable emulsions, such as turpentine liniment, without the use of surfactants. Carbopol polymers and Pemulen polymeric emulsifiers are often the thickener and emulsifier of choice in topical lotions.

POLYMERS

Poly (acrylic acid) polymers with the proprietary names Carbopol 971P, Carbopol974P Carbopol 940P were gift from Colorcon Asia, India ltd. The manufacturer states that C934 gel has the lowest cross-linking density, while that of Carbopol 971P, Carbopol 974P is intermediate and that of C940 is the highest.

Polymers used in the formulations

The Carbopol polymers used in the experiments described in this thesis were all cross linked poly (acrylic acid) polymers. The manufacturer states that the C934 gel has the lowest cross-linking density, while that of C971, C974, and is intermediate and that of C940 is the highest.

Carbopol was delivered from BF Goodrich as dry flocculated powders consisting of primary particles with an average diameter of around 0.2- 0.5m. The flocculated powders (average size 2-7 m) cannot be broken down into the ultimate particle once produced. Each primary particle contains a network of cross-linked polymer chains and will swell up to 1000 times their volume in contact with water to form a continuous gel when exposed to a suitable pH (according to manufacturer). As a vehicle for incorporation of drug and liposomes containing drug for topical delivery, adhesion gel was made. Carbopol 974P, Carbopol 971P, Carbopol 940P.

8.3.4 Preparation of Carbopol gels

1g, 2g, 3g was dispersed in demineralised water 100ml by stirring at 800rpm for 60 minutes to get 1%, 2%, 3% carbopol gel. Then, propylene glycol, 10g was added. The mixture was neutralized by drop wise on addition of 10% NaOH. Mixing was continued until a clear transparent gel appeared, while the amount of the base was adjusted to achieve gel with pH 6.5. Carbopol gels were centrifuged at 1,500-2,000 rpm before measurement in order to remove entrapped air. All gels were allowed to equilibrate for at least 16 hours at room temperature.

Incorporation of liposomes into gel

Liposomes containing zidovudine were mixed in to the 1%, 2%, 3% Carbopol gel with electrical mixer (25 rpm, 3minutes), The concentration of incorporated drug in liposomes was equivalent to 10mg.

Effects on drug release :

The rate of release of Zidovudine could be controlled by formulating the drug in different poly (acrylic acids) (PAA) with different cross-linking density, different concentrations. The rate of release from C940 was slowest, while that from C971& C974 (which has the lowest cross-linking density) was fastest than compared with 940. The polymer concentration also affected the rate of release. High concentration resulted in a slower release since there was more polymer present that the liposomes could electrostatically interact with. The polymer concentration, however, can seldom be used as an effective tool for tailoring the release rate since the formulation must have good rheological properties in order to have a long residence time at the site of drug absorption. The release was fastest from the least cross-linked polymer and slowest from polymer C940. The effect of polymer concentration was somewhat different. The release curve of 971P (1%,2%,3%), 974P(1%,2%,3%), had a quite different shape than those of 1%, 2%,3% 940P.

Table-52: Percentage drug release from 1%, 2%, 3% Carbopol 971P

S. No.	Time in hours	Drug release from 1% C971P	Drug release from 2% C971P	Drug release from 3% C971P
1.	1	12.8	10.12	6.5
2.	2	28	21.62	14
3.	4	36.5	27.5	20
4.	6	45.5	34	22.35
5.	8	54.5	43.82	30
6.	10	64	55.28	35
7.	12	73	66.13	39.5
8.	14	83.5	74.21	46.5
9.	16	88.26	82.56	52
10.	18	94.68	84.28	57.5
11.	20	98.26	85.34	59.5
12.	22	98.56	85.68	65
13.	24	98.89	85.72	66.5

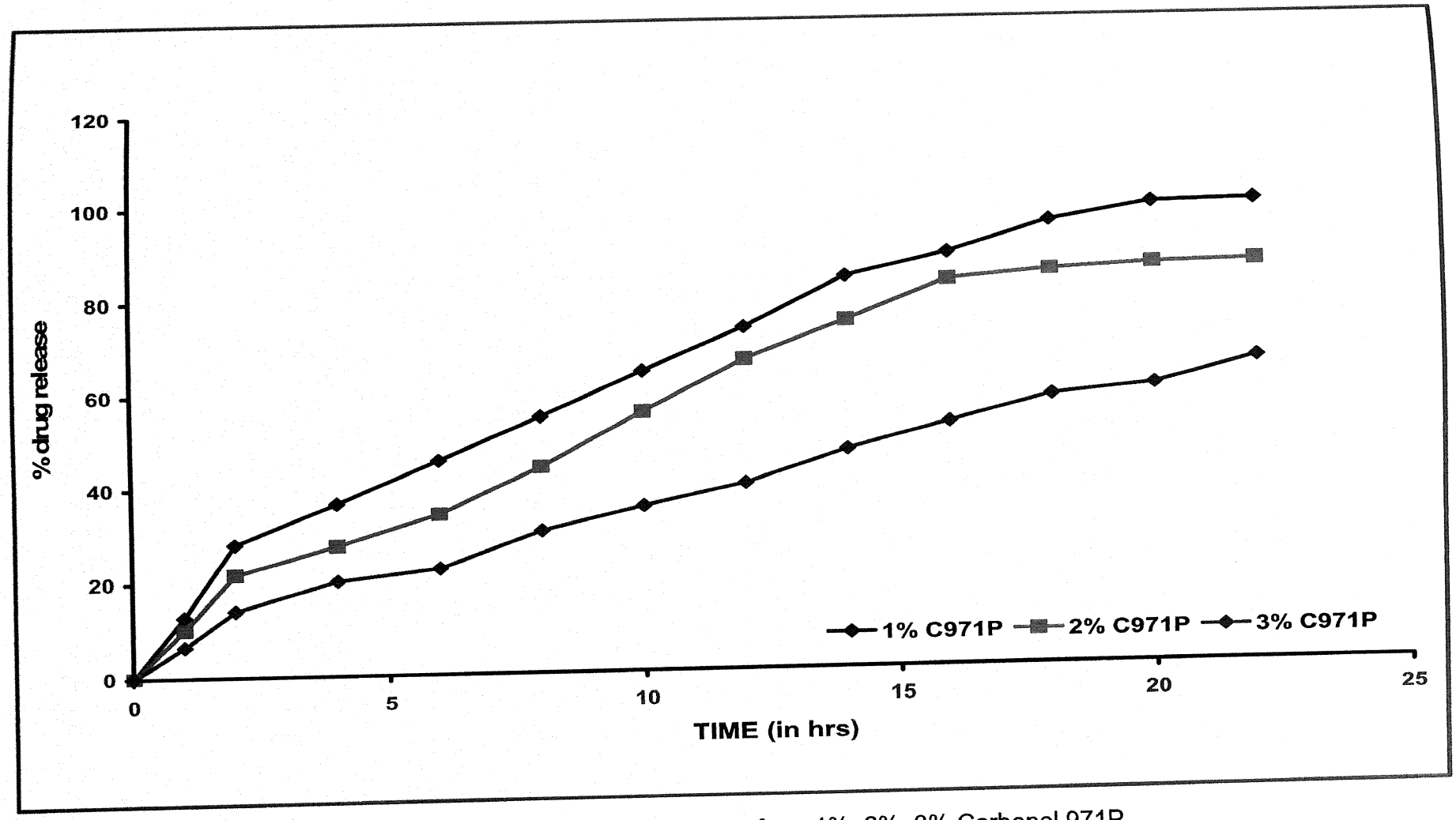


Figure-102 : Percentage drug release from 1%, 2%, 3% Carbopol 971P

Table-53: Percentage drug release model from Carbopol C971P.

Model		Percentage release from Carbopol C971P		
		1%Carbopol C971P	2%Carbopol C971P	3%Carbopol C971P
Zero Order	R	0.9504	0.9607	0.9872
	k	2.8188	2.4602	1.6997
	SSQ	467	307	51
1st Order	R	0.9883	0.9838	0.9961
	k	-0.0393	-0.0325	-0.0204
	SSQ	110	94	20
Matrix	R	0.9864	0.9735	0.9653
	k	11.5662	10.0447	6.8784
	SSQ	130	209	137
Peppas	R	0.9933	0.9917	0.9941
	k	7.3706	5.4706	3.5163
	SSQ	39	75	13
Hix Crow	R	0.9795	0.9781	0.9942
	k	-0.0117	-0.0098	-0.0064
	SSQ	188	141	26
		PEPPAS	PEPPAS	PEPPAS

Table-54: Average percentage drug release from CarbopolC971P-1%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	467	110	130	39	188
1	1	6.411	12.904	6.551	26.577	0.921	8.727
2	2	14.186	73.079	43.979	4.713	6.165	54.072
3	4	18.682	54.866	17.172	19.804	0.010	28.464
4	6	23.523	43.704	6.395	23.114	0.691	15.785
5	8	28.494	35.334	2.345	17.806	1.025	9.266
6	10	33.897	32.595	2.006	7.175	0.120	8.079
7	12	39.075	27.564	2.232	0.982	0.163	7.315
8	14	44.907	29.634	6.810	2.656	4.189	12.252
9	16	48.150	9.301	2.232	3.555	1.683	3.926
10	18	52.225	2.211	2.368	9.945	2.379	2.429
11	20	54.938	2.066	0.277	10.320	0.320	0.001
12	22	56.081	35.187	3.148	3.352	3.463	7.918
13	24	57.227	108.643	14.531	0.319	17.449	29.318

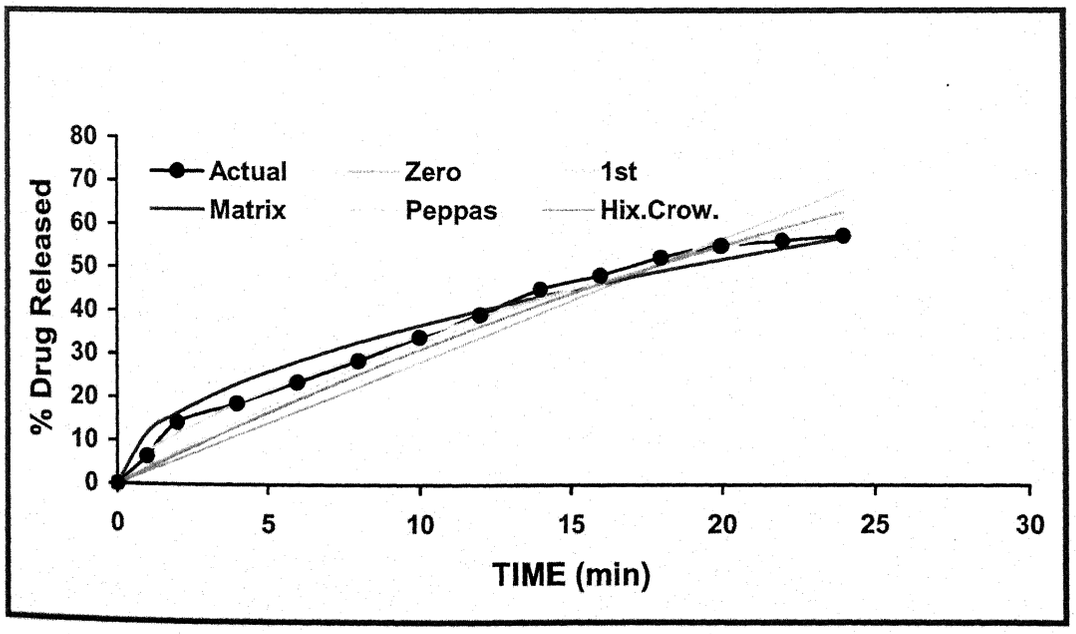


Figure-103: Average percentage drug release from CarbopolC971P-1%

Table-55: Average percentage drug release from CarbopolC971P-2%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	307	94	209	75	141
1	1	5.050	6.708	3.433	24.946	0.177	4.530
2	2	10.948	36.328	21.672	10.613	3.692	26.642
3	4	14.075	17.928	3.558	36.176	0.668	7.455
4	6	17.662	8.414	0.003	48.202	5.283	0.964
5	8	22.619	8.632	0.074	33.540	3.808	0.676
6	10	29.034	19.647	1.666	7.453	0.027	5.449
7	12	34.987	29.862	7.271	0.036	4.220	12.910
8	14	39.702	27.666	9.933	4.487	8.356	14.590
9	16	44.599	27.422	16.453	19.540	16.484	19.608
10	18	46.308	4.100	4.095	13.629	4.702	4.176
11	20	47.653	2.404	0.019	7.461	0.001	0.264
12	22	48.707	29.344	5.610	2.537	5.375	10.081
13	24	49.624	88.734	20.517	0.172	22.195	33.539

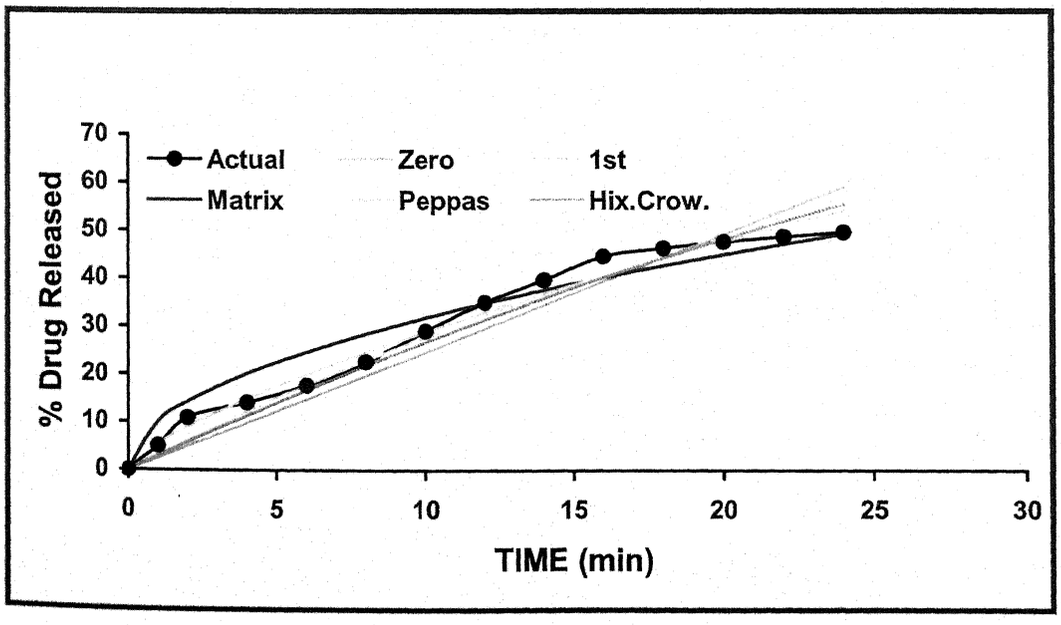


Figure-104: Average percentage drug release from CarbopolC971P-2%

Table-56: Average percentage drug release from CarbopolC971P-3%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	51	20	137	13	26
1	0	0.000	0.000	0.000	0.000	-	0.000
2	1	3.260	2.434	1.544	13.094	0.066	1.841
3	2	7.098	13.682	9.639	6.913	1.480	11.001
4	4	10.250	11.909	5.864	12.300	0.169	7.746
5	6	11.574	1.895	0.004	27.816	2.951	0.269
6	8	15.631	4.137	0.346	14.620	0.680	1.165
7	10	18.500	2.261	0.004	10.568	0.845	0.298
8	12	21.040	0.414	0.428	7.772	1.425	0.051
9	14	24.967	1.373	0.021	0.592	0.002	0.231
10	16	28.143	0.899	0.102	0.396	0.381	0.275
11	18	31.412	0.670	0.499	4.972	1.884	0.562
12	20	32.991	1.005	0.233	4.972	0.258	0.396
13	22	36.336	1.117	0.042	16.591	2.167	0.025
14	24	37.748	9.264	0.870	16.413	0.313	2.347

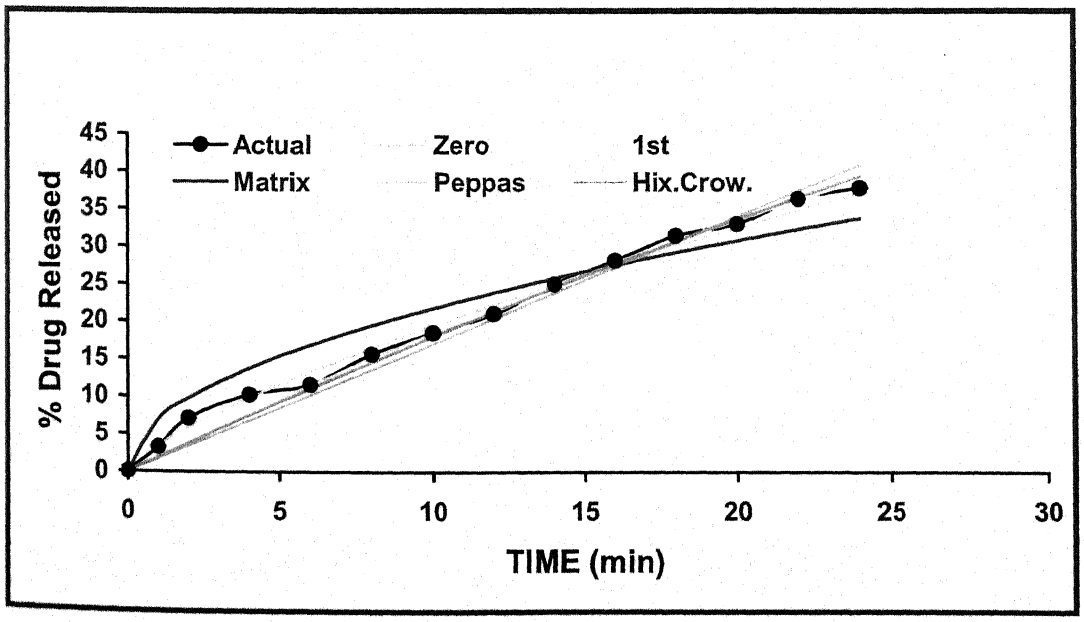


Figure-105: Average percentage drug release from CarbopolC971P-3%

Table-57: Percentage drug release from 1%, 2%, 3% Carbopol 974P

S. No.	Time in hours	Drug release from 1% C974P	Drug release from 2% C974P	Drug release from 3% C974P
1.	0	0	0	0
2.	1	10.18	9.24	6.2
3.	2	22.46	18.22	11.12
4.	4	30.45	25	15.24
5.	6	36.24	31.2	19.68
6.	8	45.25	35.2	24.67
7.	10	53.68	40.21	28.45
8.	12	60.35	46.23	32.65
9.	14	68.54	51.36	37.9
10.	16	78.69	57.55	43.98
11.	18	82.69	62.44	48.56
12.	20	83.42	64.81	54.26
13.	22	83.99	68.57	58.35
14.	24	84.01	70.23	61.2

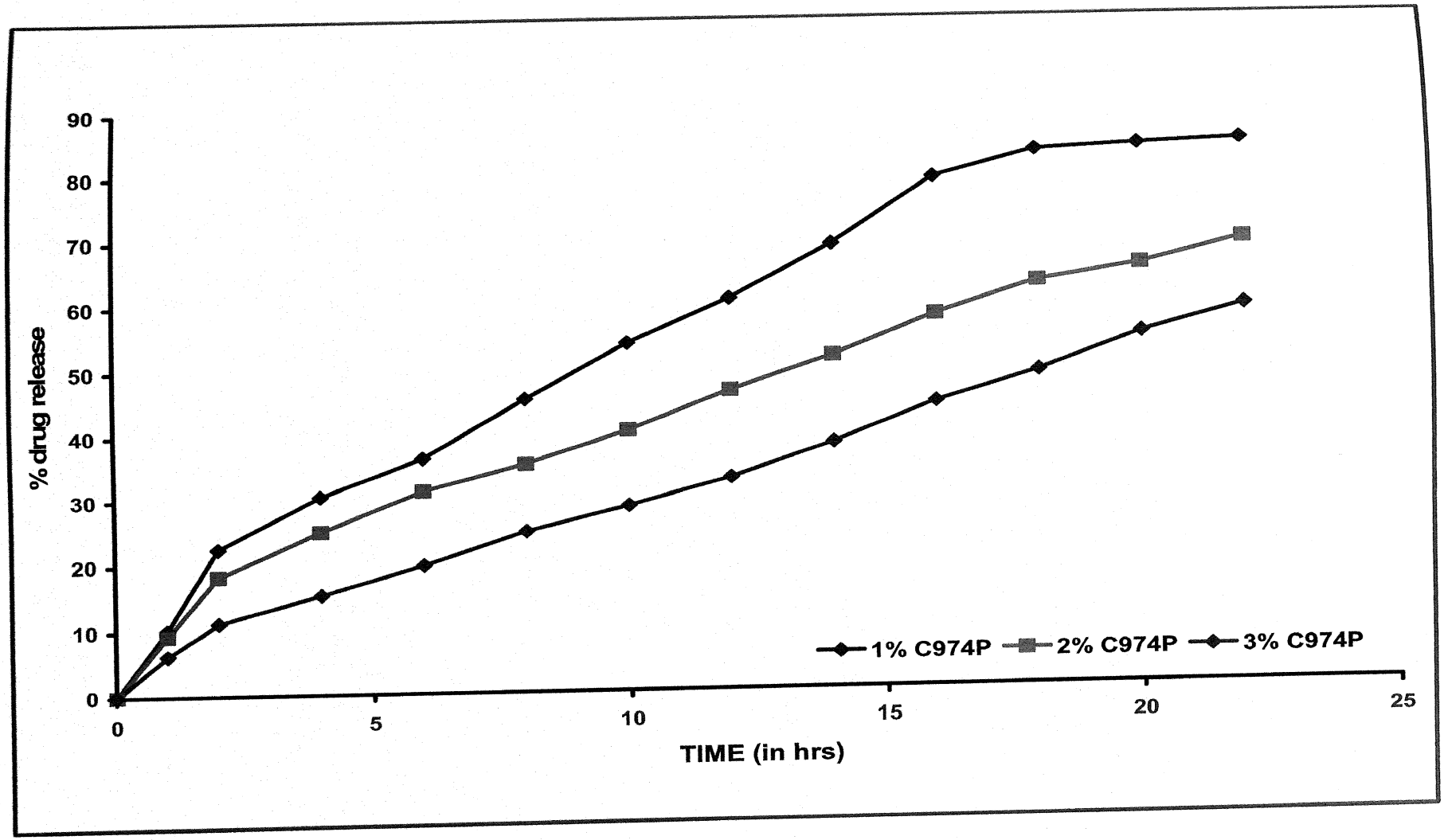


Figure-106: Percentage drug release from 1%, 2%, 3% Carbopol 974P

Table-58: Percentage drug release model from Carbopol C974P

Model		Percentage release from Carbopol C974P		
		1%Carbopol C974P	2%Carbopol C974P	3%Carbopol C974P
Zero Order	R	0.9580	0.9648	0.9945
	k	2.3926	1.8759	1.4789
	SSQ	297	150	18
1st Order	R	0.9848	0.9872	0.9964
	k	-0.0313	-0.0229	-0.0173
	SSQ	94	66	13
Matrix	R	0.9808	0.9838	0.9505
	k	9.7905	7.6686	5.9457
	SSQ	137	70	156
Peppas	R	0.9932	0.9958	0.9939
	k	5.8072	5.0537	2.9621
	SSQ	41	8	28
Hix Crow	R	0.9782	0.9813	0.9965
	k	-0.0095	-0.0071	-0.0055
	SSQ	141	88	13
		PEPPAS	PEPPAS	HIX.CROWEL

Table-59: Average percentage drug release from CarbopolC974P-1%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	297	94	137	41	141
1	1	5.090	7.277	4.045	22.092	0.514	5.138
2	2	11.330	42.832	27.735	6.331	3.779	32.907
3	4	15.589	36.219	14.665	15.940	0.175	21.294
4	6	18.764	19.434	2.737	27.225	1.754	6.852
5	8	23.622	20.086	2.211	16.559	0.802	6.290
6	10	28.290	19.045	2.054	7.131	0.106	5.797
7	12	32.139	11.747	0.717	3.157	0.108	2.838
8	14	36.876	11.424	2.012	0.059	0.563	4.172
9	16	42.620	18.822	10.555	11.957	8.958	12.919
10	18	45.414	5.510	5.601	15.026	5.850	5.665
11	20	46.602	1.561	0.010	7.940	0.124	0.068
12	22	47.718	24.200	4.108	3.226	2.852	7.827
13	24	48.560	78.546	17.910	0.355	15.336	29.566

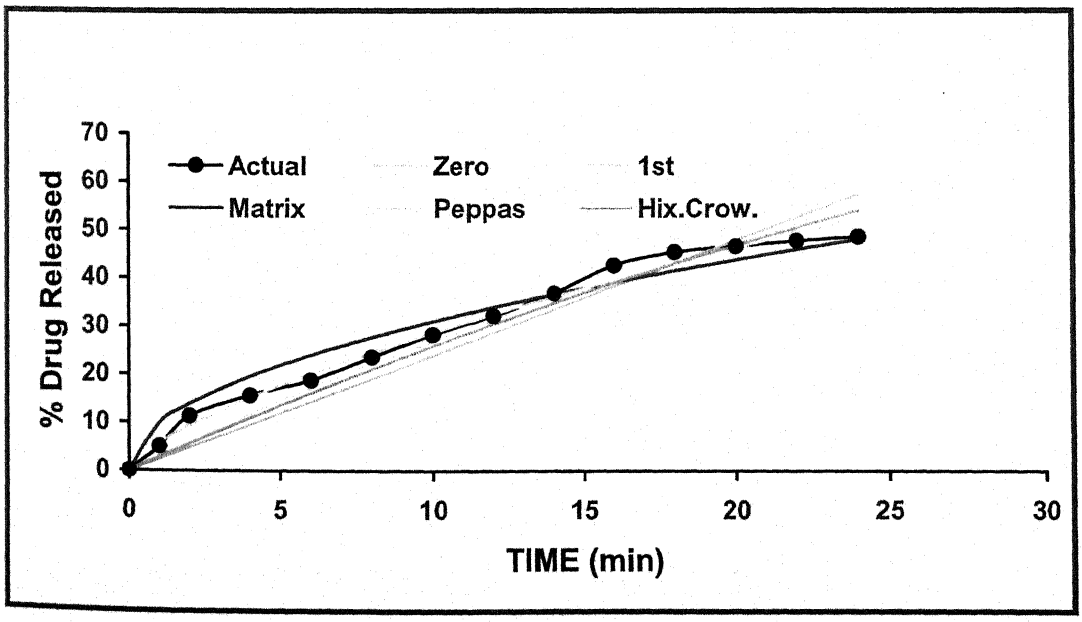


Figure-107: Average percentage drug release from CarbopolC974P-1%

Table-60: Average percentage drug release from CarbopolC974P-2%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	150	66	70	8	88
1	1	4.625	7.556	5.576	9.266	0.184	6.258
2	2	9.203	29.716	22.349	2.697	1.591	24.873
3	4	12.827	28.344	16.619	6.300	0.121	20.395
4	6	16.189	24.346	11.255	6.733	0.005	15.218
5	8	18.510	12.270	3.147	10.115	1.215	5.574
6	10	21.332	6.621	0.756	8.517	1.827	2.067
7	12	24.765	5.085	0.553	3.238	0.612	1.535
8	14	27.758	2.237	0.112	0.875	0.241	0.505
9	16	31.343	1.765	0.452	0.446	0.274	0.782
10	18	34.371	0.366	0.357	3.369	1.190	0.372
11	20	36.215	1.695	0.272	3.687	0.324	0.551
12	22	38.671	6.752	0.807	7.298	0.545	1.902
13	24	40.219	23.060	4.222	7.024	0.005	7.955

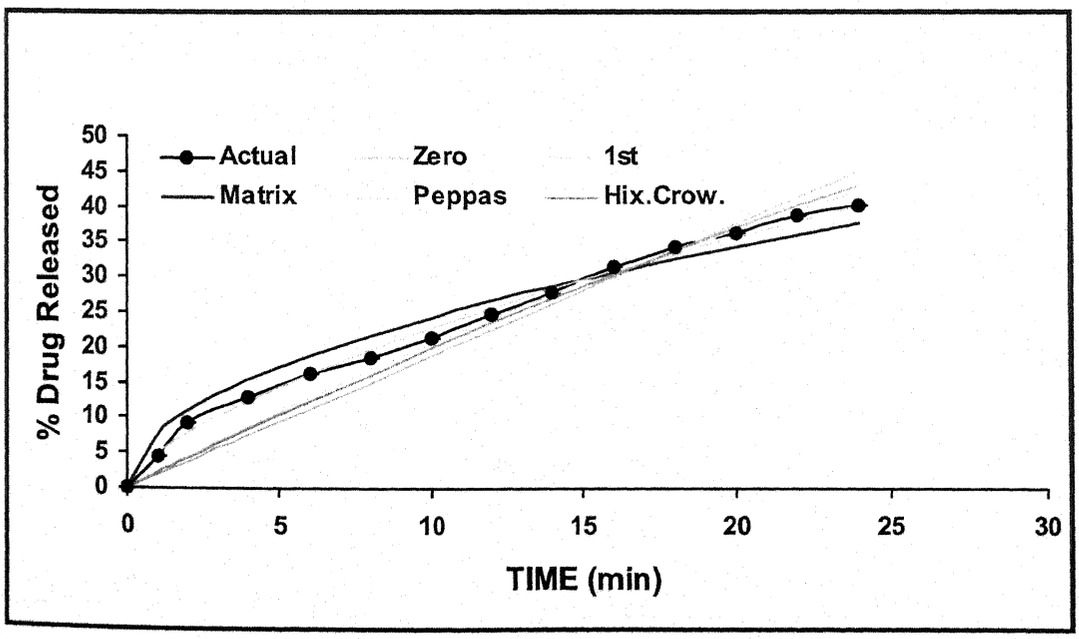


Figure-108: Average percentage drug release from CarbopolC974P-2%

Table-61: Average percentage drug release from CarbopolC974P-3%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	18	13	156	28	13
1	0	0.000	0.000	0.000	0.000	-	0.000
2	1	3.119	2.691	1.967	7.989	0.025	2.213
3	2	5.630	7.143	4.957	7.718	0.439	5.686
4	4	7.809	3.586	1.248	16.667	0.273	1.928
5	6	10.170	1.681	0.091	19.312	1.222	0.415
6	8	12.855	1.049	0.007	15.697	1.251	0.087
7	10	15.029	0.058	0.758	14.239	2.179	0.248
8	12	17.421	0.106	1.798	10.083	2.215	1.009
9	14	20.358	0.120	1.368	3.569	0.733	0.809
10	16	23.748	0.007	0.203	0.001	0.097	0.075
11	18	26.496	0.015	0.080	1.613	0.823	0.051
12	20	29.832	0.065	0.314	10.508	4.626	0.225
13	22	32.402	0.018	0.523	20.374	7.188	0.224
14	24	34.430	1.129	0.182	28.114	7.378	0.000

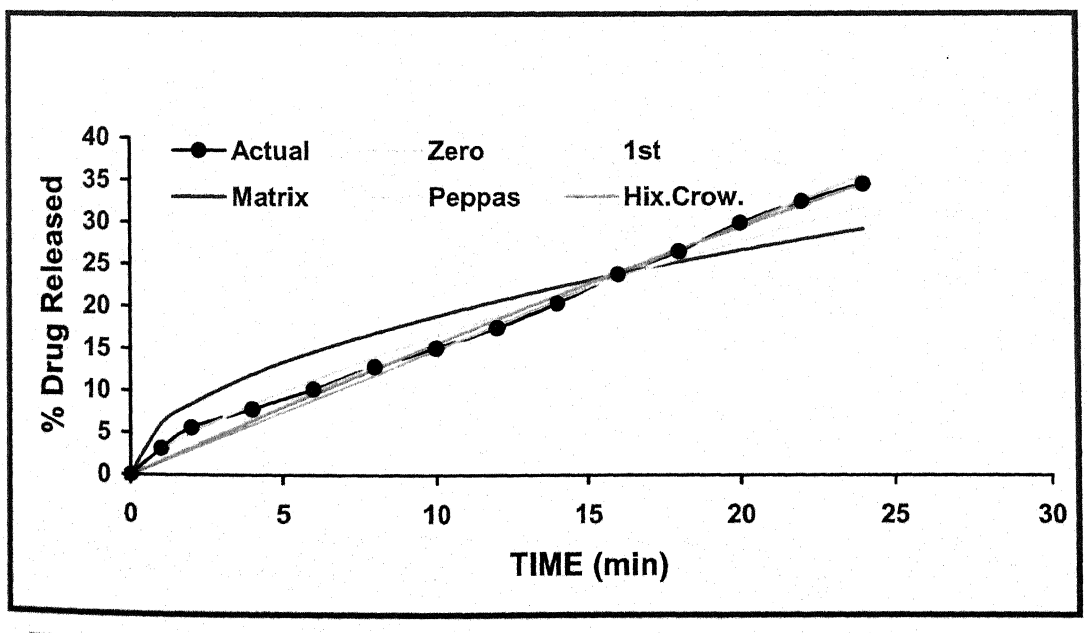


Figure- 109: Average percentage drug release from CarbopolC974P-3%

Table-62: Drug release from Carbopol 940P

S. No.	Time in hours	Drug release from 1% C940P	Drug release from 2% C940P	Drug release from 3% C940P
1.	0	0	0	0
2.	1	8.2	5.4	1.6
3.	2	11.5	8.6	6
4.	4	14.3	11.56	9.3
5.	6	17.24	15.23	12.3
6.	8	21.12	18.96	13.8
7.	10	24.28	21.99	16.7
8.	12	27.18	25.6	19.6
9.	14	32.56	29.4	22.8
10.	16	37	33.3	27
11.	18	41	38.25	28.98
12.	20	42.58	39.87	30.1
13.	22	43.56	40.35	31.4
14.	24	43.59	40.87	32.7

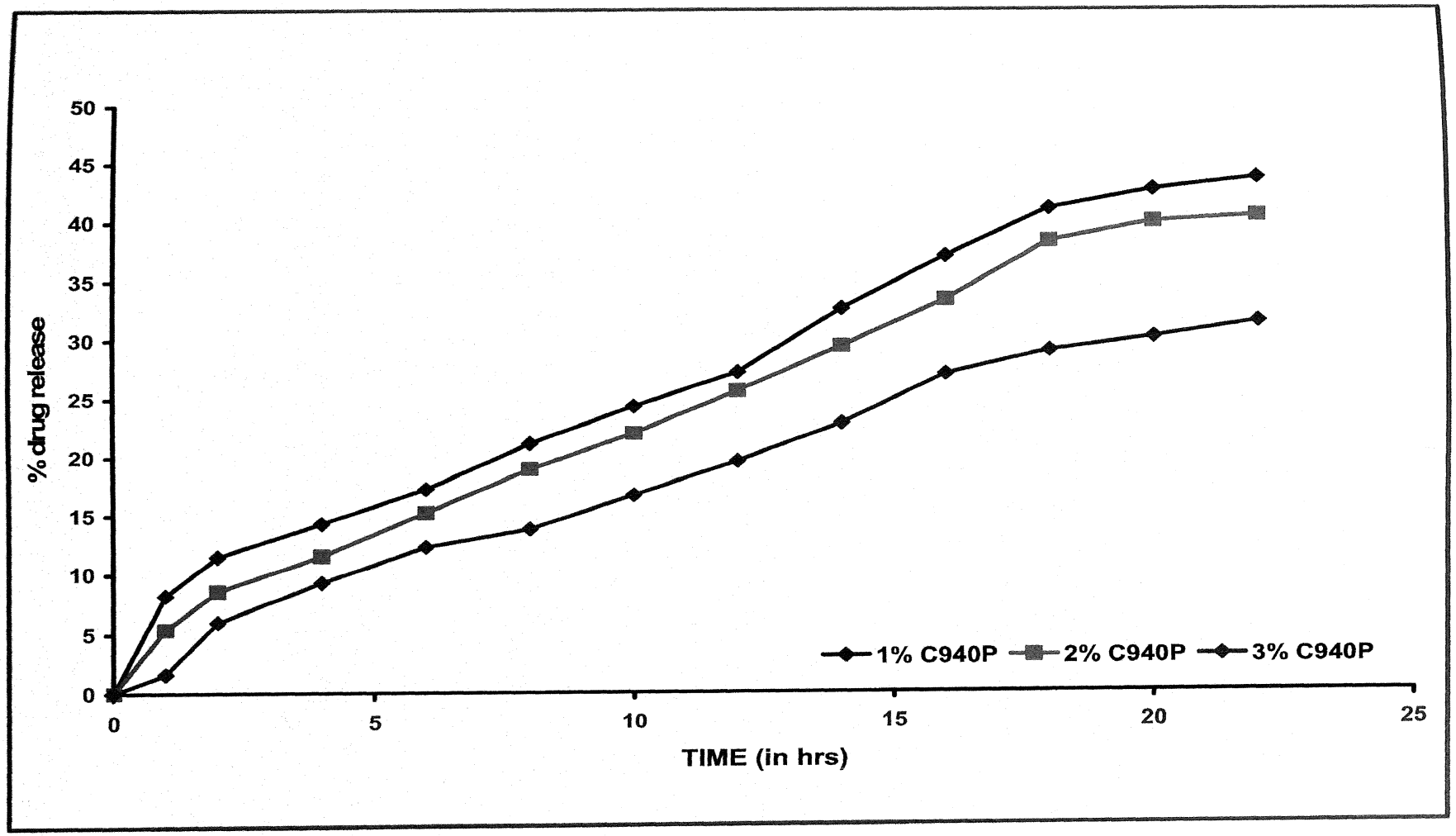


Figure-110: Drug release from Carbopol 940P

Table-63: Percentage drug release model from Carbopol C940P

Model		Percentage release from Carbopol C940P		
		1%Carbopol C940P	2%Carbopol C940P	3%Carbopol C940P
Zero Order	R	0.9702	0.9841	0.9892
	k	1.1818	1.0860	0.8406
	SSQ	52	26	11
1st Order	R	0.9818	0.9906	0.9933
	k	-0.0133	-0.0121	-0.0091
	SSQ	34	15	7
Matrix	R	0.9735	0.9653	0.9593
	k	4.8141	4.3989	3.3933
	SSQ	46	55	41
Peppas	R	0.9855	0.9939	0.9835
	k	3.5788	2.4976	1.1504
	SSQ	23	11	6
Hix Crow	R	0.9784	0.9888	0.9921
	k	-0.0043	-0.0039	-0.0030
	SSQ	39	18	8
		PEPPAS	PEPPAS	ISTORDER

Table-64: Average percentage drug release from CarbopolC940 P-1%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	52	34	46	23	39
1	0	0.000	0.000	0.000	0.000	-	0.000
2	1	4.103	8.532	7.739	0.506	0.275	8.012
3	2	5.841	12.090	10.346	0.936	0.173	10.938
4	4	7.351	6.883	4.715	5.187	0.759	5.415
5	6	8.961	3.497	1.673	8.015	2.327	2.219
6	8	11.078	2.636	0.975	6.442	1.915	1.445
7	10	12.881	1.130	0.186	5.487	1.867	0.412
8	12	14.563	0.146	0.034	4.465	1.772	0.000
9	14	17.525	0.959	0.292	0.238	0.008	0.468
10	16	20.062	1.330	0.809	0.650	1.376	0.965
11	18	22.435	1.352	1.325	4.044	4.679	1.338
12	20	23.628	0.000	0.077	4.405	4.132	0.037
13	22	24.556	2.086	0.649	3.902	2.854	1.000
14	24	24.991	11.373	5.423	1.980	0.809	6.987

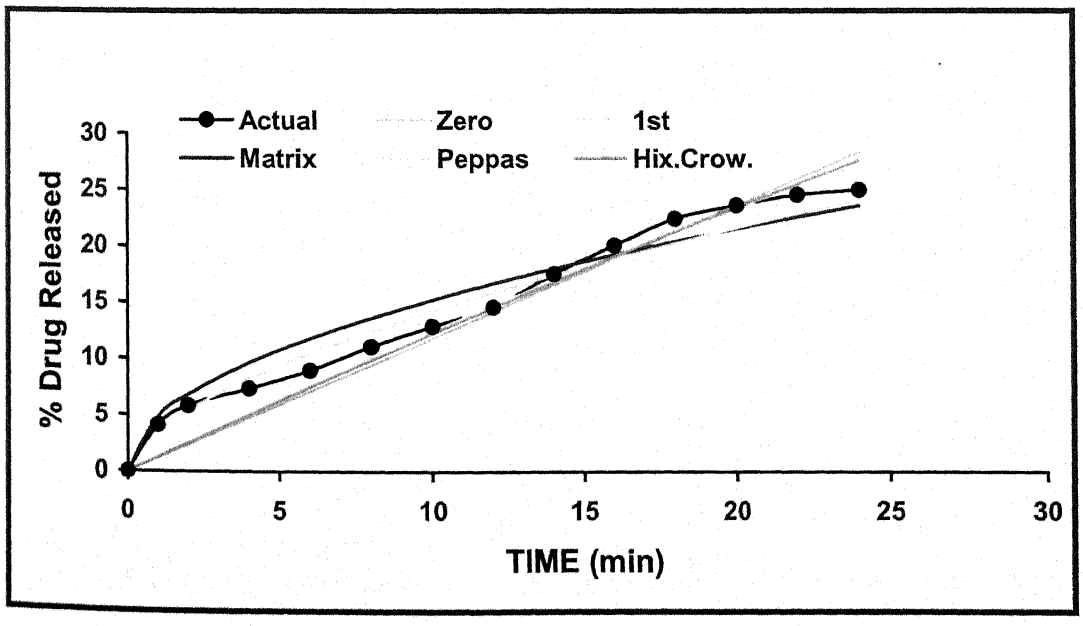


Figure-111: Average percentage drug release from CarbopolC940 P-1%

Table-65: Average percentage drug release from CarbopolC940P-2%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	26	15	55	11	18
1	1	2.738	2.729	2.357	2.759	0.058	2.483
2	2	4.358	4.780	3.869	3.470	0.090	4.174
3	4	5.930	2.514	1.450	8.227	0.445	1.783
4	6	7.872	1.838	0.754	8.429	0.795	1.069
5	8	9.891	1.446	0.440	6.509	0.688	0.714
6	10	11.592	0.535	0.038	5.377	0.889	0.139
7	12	13.672	0.410	0.024	2.453	0.325	0.100
8	14	15.776	0.327	0.037	0.467	0.008	0.100
9	16	18.037	0.436	0.188	0.195	0.378	0.258
10	18	20.819	1.615	1.551	4.647	3.602	1.575
11	20	22.024	0.092	0.278	5.530	2.735	0.211
12	22	22.664	1.509	0.504	4.125	0.786	0.755
13	24	23.330	7.475	3.520	3.168	0.034	4.567

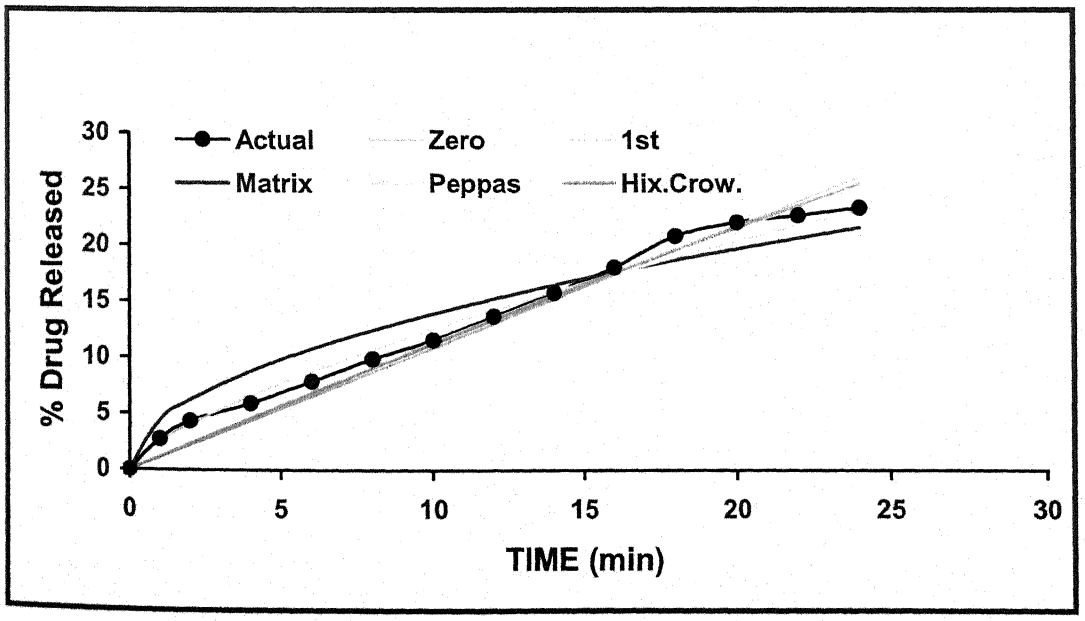


Figure-112: Average percentage drug release from CarbopolC940P-2%

Table-66: Average percentage drug release from CarbopolC940P-3%

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	11	7	41	6	8
1	0	0.000	0.000	0.000	0.000	-	0.000
2	1	0.791	0.002	0.014	6.772	0.129	0.009
3	2	3.035	1.832	1.503	3.112	0.788	1.613
4	4	4.741	1.900	1.337	4.185	0.539	1.518
5	6	6.320	1.628	0.982	3.970	0.300	1.182
6	8	7.195	0.221	0.024	5.773	0.080	0.068
7	10	8.788	0.146	0.004	3.773	0.125	0.029
8	12	10.405	0.101	0.001	1.823	0.135	0.016
9	14	12.182	0.171	0.035	0.265	0.037	0.069
10	16	14.538	1.184	0.906	0.930	0.339	0.993
11	18	15.791	0.436	0.413	1.945	0.075	0.421
12	20	16.641	0.029	0.002	2.149	0.176	0.007
13	22	17.587	0.822	0.365	2.791	1.004	0.488
14	24	18.551	2.635	1.252	3.715	2.409	1.628

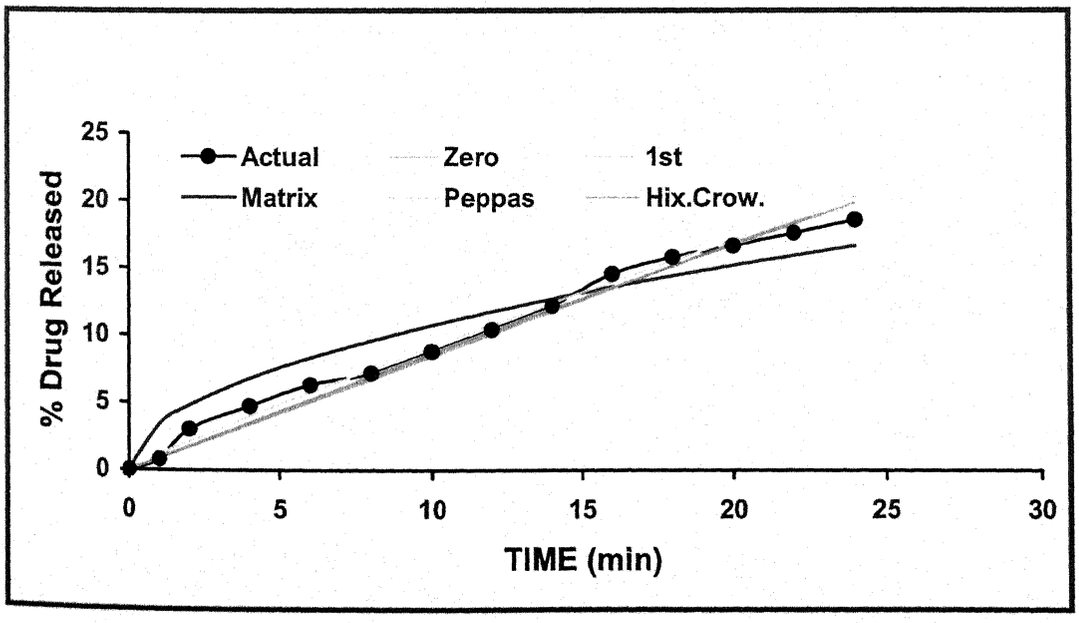


Figure-113: Average percentage drug release from CarbopolC940P-3%

Stability studies of conventional liposomes and the liposomal gel of the zidovudine

The ability of vesicles to retain the drug (Drug retentive behavior) was assessed by keeping the liposomal gel of Carbopol at different temperature conditions, i.e. 4°C, Room temperature, 37°C for a period of three months. Through out the study the liposomal Carbopol gel were stored in aluminium foil sealed glass vials. The samples were taken every week and release studies were conducted to find out the drug content spectrophotometricaly at 267 nm.

Table-67: Stability of 1%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

S. No.	Time in months	% drug retained in 4°C	% drug retained in R.T	% drug retained in 37°C
1	Initial	100	100	100
2	1	98.24	98.01	93.48
3	2	96.01	94.41	86.32
4	3	95.41	89.7	78.65

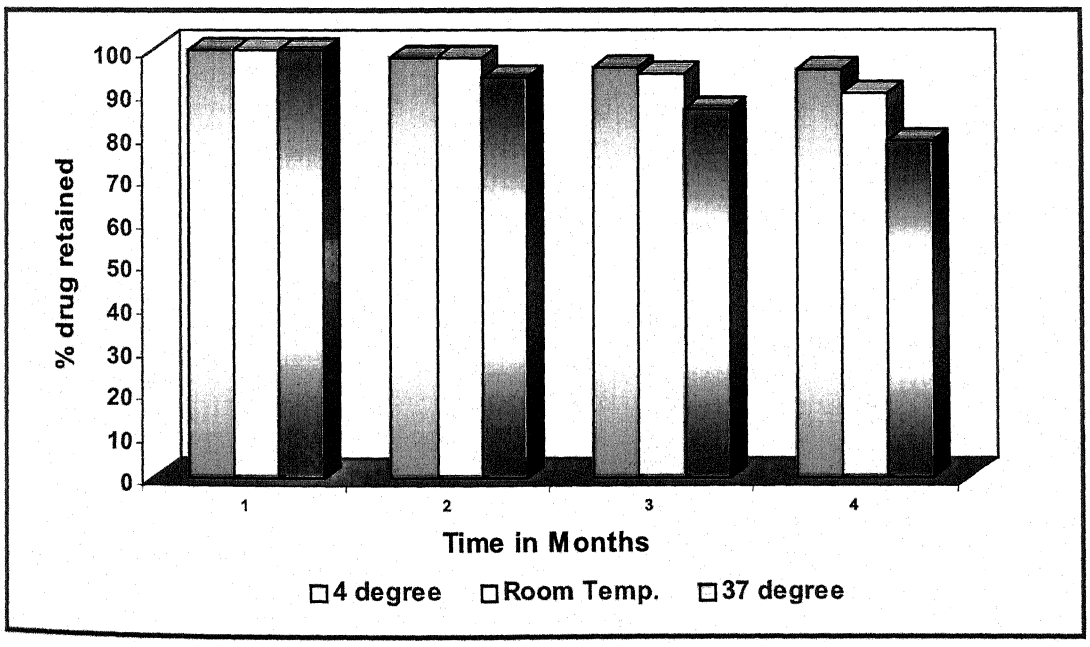


Figure-114(a) : Stability of 1%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

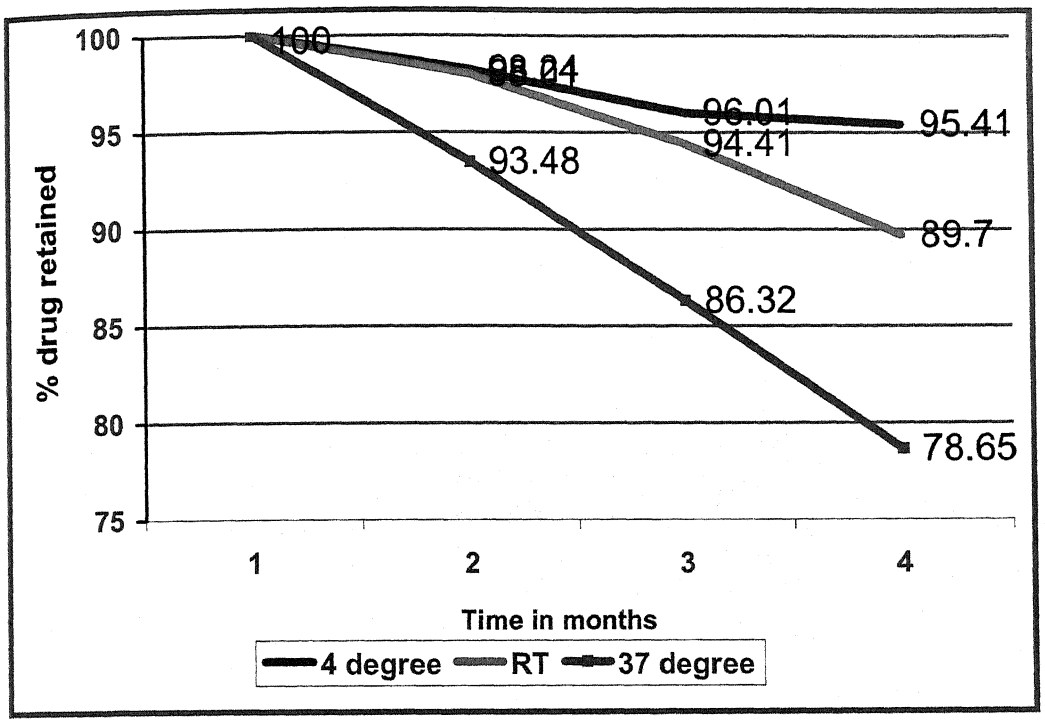


Figure-114(b) : Stability of 1%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

Table-68: Stability of 2% Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

S. No.	Time in months	% drug retained in 4°C	% drug retained in R.T	% drug retained in 37°C
1.	Initial	100	100	100
2.	1	99.18	97.84	94.25
3.	2	98.02	95.41	88.46
4.	3	96.41	91.7	81.23

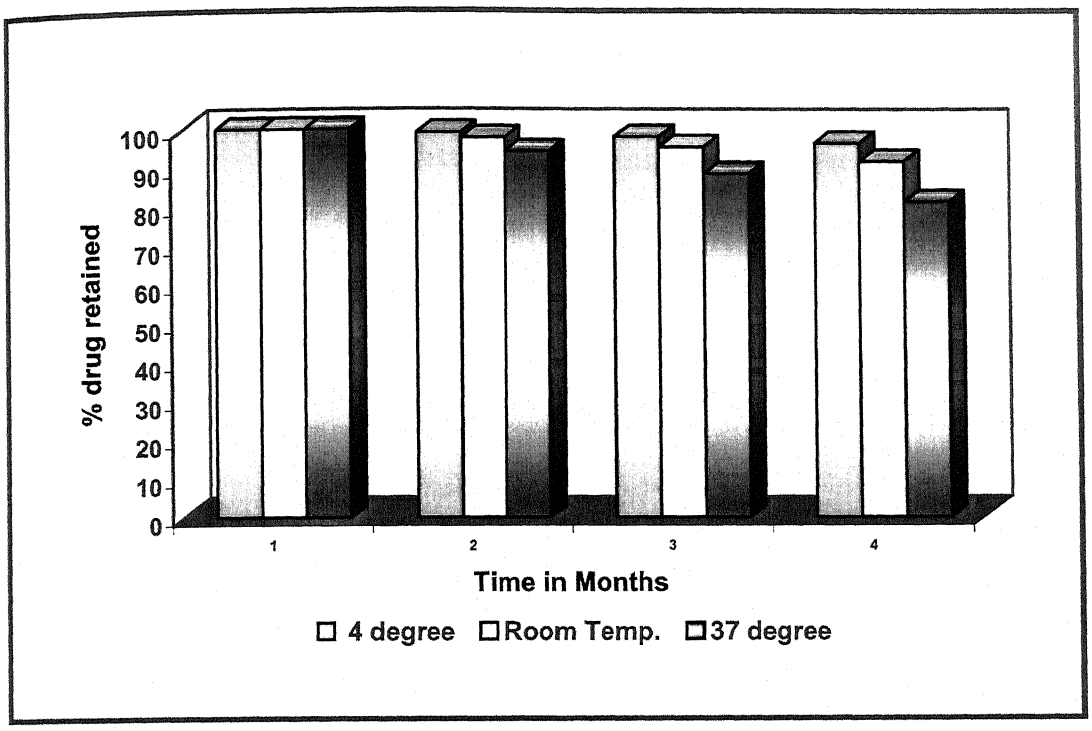


Figure-115: Stability of 2%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

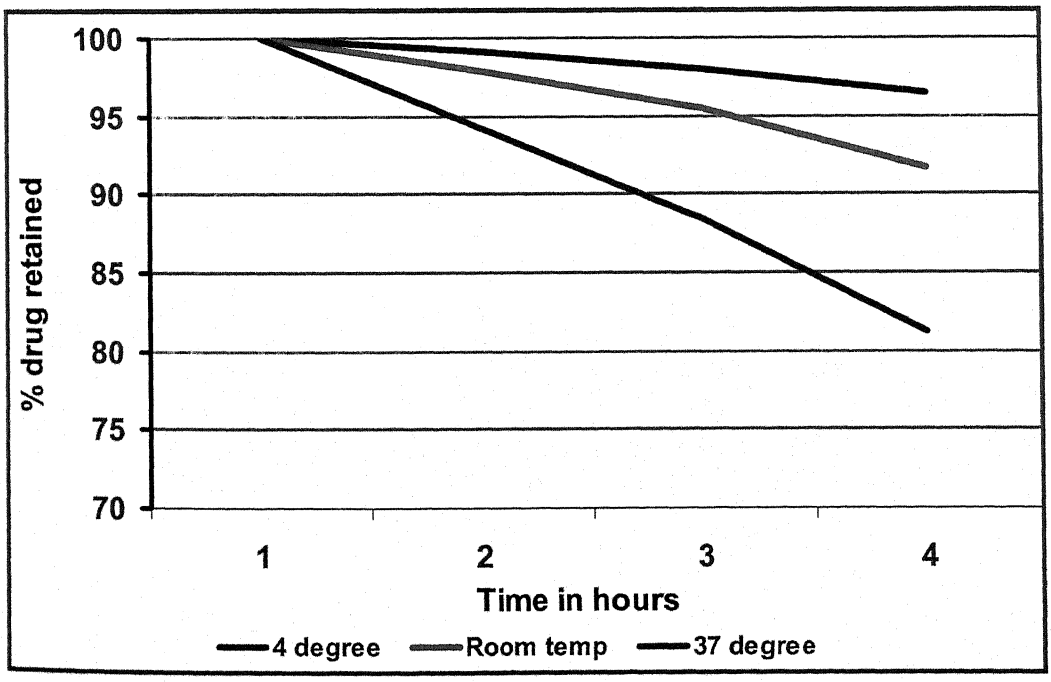


Figure-116: Stability of 2%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

Table-69: Stability of 3%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.

S. No.	Time in months	% drug retained in 4°C	% drug retained in R.T	% drug retained in 37°C
1.	Initial	100	100	100
2.	1	99.01	97.86	94.22
3.	2	98.23	96.71	90.4
4.	3	96.89	92.53	86.14

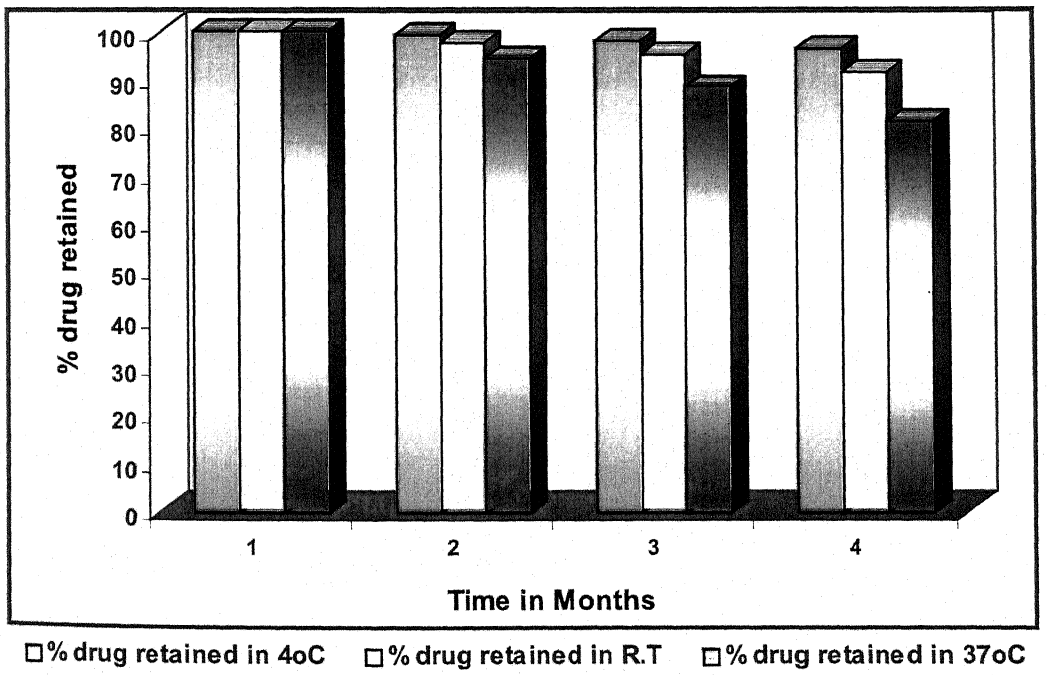
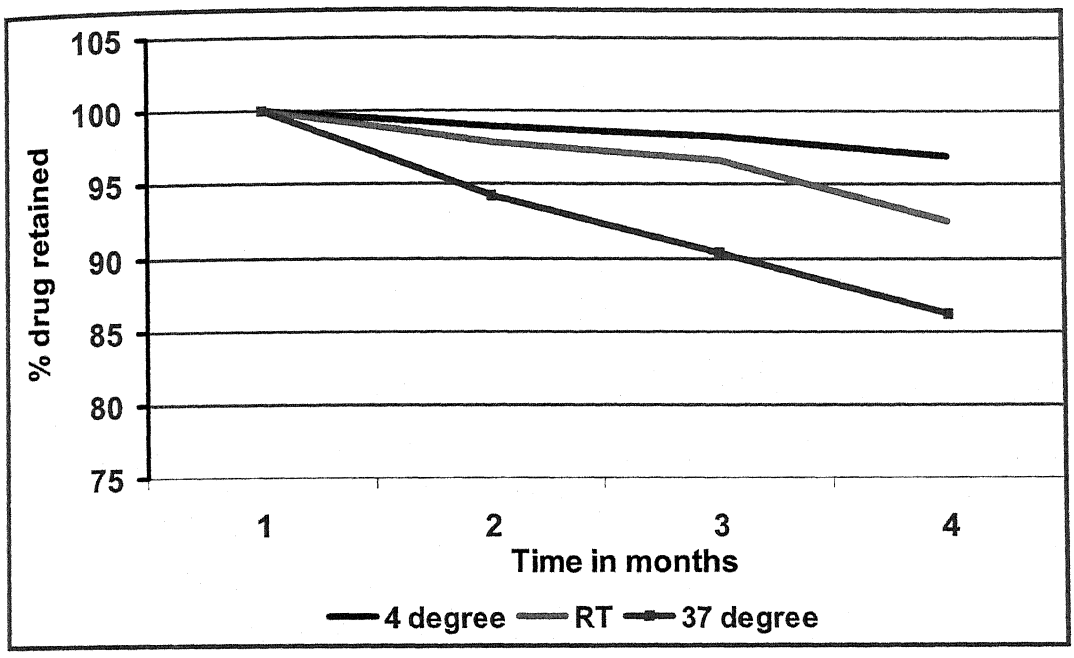


Figure-117(a): Stability of 3%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three month

Figure-117(b): Stability of 3%Carbopol liposomal gel containing zidovudine at 4°C, room temperature and 37°C after three months.



Chapter-9

HPMC
(Hydroxy Propyl Methyl Cellulose)

HPMC

9.1 INTRODUCTION

Hydrophilic polymer gel matrix systems are widely used in controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost effectiveness. The hydrophilic polymer matrix system consists of hydrophilic polymer, drug, liposomes distributed throughout the gel matrix. This dynamic system is dependent on polymer wetting, hydration for controlled release of drug. At the same time, liposome substances will also wet and diffuse out of the gel matrix, whereas insoluble substance or drug substances will be held in place until the surrounding polymer. Hydroxypropyl methyl cellulose (HPMC), which is commonly used in hydrophilic matrix drug delivery systems, is mixed alkyl hydroxyl alkyl cellulose ether containing methoxyl and hydroxypropyl groups. The hydration rate of HPMC depends on the nature of these substituents, such as the molecular structure and the degree of substitution. Specifically, the hydration rate of HPMC increases with an increase in the hydroxypropyl content. The solubility of HPMC is pH independent. HPMC can be slowly dissolved in cold water to form a viscous solution, but is not very soluble in hot water. Additionally, it can be dissolved in most polar organic solvents or binary systems of methylene chloride or chloroform and alcohol[148]. It is practically insoluble in pure chloroform, ethanol, or ether. Therefore, the recommended method to prepare HPMC aqueous solutions is to first thoroughly disperse and hydrate the powder in a portion of hot water heated above 90°C with vigorous stirring to prevent lumping. Complete solubilisation is then accomplished by adding the remaining portion as cold water (two thirds of the total volume) to lower the temperature of the dispersion. As the temperature is lowered, HPMC becomes water soluble, resulting in increased viscosity ("hot/cold" techniques). Increasing the molecular weight distribution of the polymer or the concentration of the polymer or decreasing the temperature of the solution can increase the viscosity of the aqueous solution. In gel preparation, quantification of the drug substance (i.e., content uniformity) in the drug product is a mandatory requirement for release. However, extracting a hydrophilic

drug substance from gelling HPMC tablets is very much easy because of the hydrophilic nature of the drug substance and the gelling properties of HPMC polymer.

The objective of this study was to develop a suitable method for the recovery of a hydrophilic drug in liposomes from controlled-release gel containing HPMC. To develop a method to recover a hydrophilic drug from controlled-release gels containing HPMC, Zidovudine-was chosen as the model drug. It has a rapid onset of action coupled with a relatively short half-life, and is indicated for managing HIV. The drug substance occurs as a white to off-white crystalline powder.

9.2 GENERAL IDENTIFICATION OF HPMC

Chemical Names:

Hydroxypropylmethyl cellulose, 2-hydryoxypropyl ether of methyl cellulose

Other Name(s):

Propylene glycol ether of methylcellulose, 2- hydroxypropyl methyl ether, modified cellulose, hypromellose, HPMC, MHPC, carbohydrate gum

Trade Name(s):

Benecel®MP643, Isopto-Tears; Methopt; Poly-Tears; Tears Naturale, Methocel E, Methocel F, Methocel K Methopt, Pharmacoat ®/Metolose

Composition: C56H108O30

Properties:

White to off-white fibrous powder or granules, swells in water to produce a viscous colloidal solution, non-ionic. Dissolves slowly in cold water, insoluble in hot water, soluble in most polar solvents, insoluble in anhydrous alcohol, ether, and chloroform. Aqueous solutions are surface active, form films upon drying and undergo reversible transformation from sol to gel upon heating and cooling[149]. The cellulose ethers are manufactured by a reaction of purified cellulose with alkylating reagents (methyl chloride) in presence of a base, typically sodium hydroxide and an inert diluent. The addition of the base in combination with water activates the cellulose matrix by disrupting the crystalline structure and increasing the access for the alkylating agent and promotes the etherification reaction. This activated matrix is

called alkali cellulose[150]. During the manufacture of HPMC alkali cellulose reacts with methyl chloride to produce methyl cellulose and sodium chloride. After this reaction, MC and HPMC are purified in hot water, where they are insoluble. Drying and grinding completes the process. Cellulose quality is measured by the content of alpha-cellulose, which is that portion insoluble in 18% alkali. Highly purified forms (over 99% alpha cellulose) are used to make the derivatives such as the cellulose gums, including sodium carboxymethylcellulose, methylcellulose and hydroxypropylmethylcellulose.

Specific Uses:

Food uses include as emulsifier, thickening agent, stabilizer, gellant, film former, protective colloid, fat barrier, suspending agent in food products; including bakery goods, ice cream, breading, dressing, salad dressings, sauce mixes. The gels produced from the cellulose derivatives have desirable fat like functional properties, including creaminess, fat like mouth-feel, stability, texture modification, increased viscosity and glossy appearance of high fat emulsions[151]. HPMC is preferred for oil emulsions, including mineral oil, vitamin A, olive, soya and flavor oils[152]. In baked products they improve tenderness and extend shelf life. A major use is in non dairy whipped toppings, also used in as emulsifiers in hand creams and lotions. They are resistant to bacterial decomposition. Methyl cellulose is sometimes called modified vegetable gum, HPMC is occasionally called carbohydrate gum[153]. HPMC has many pharmaceutical uses, as a drug carrier, a coating agent, a tabletting agent, an emulsifier in ointments. It is also used in ophthalmic solutions and as a slow release agent. It is widely used in personal care products as a thickening agent and foam stabilizer. The petitioned use is as an ingredient of hard capsules used for encapsulating powdered herbs. These are considered to be "vegetable" capsules, as they are an alternative to gelatin[154].

Action:

Methyl celluloses (MC and HPMC) have properties known as reversible thermal gelation that is the basis for many applications- they form gels when heated

but return to solubility when cooled. They are used to reduce the amount of fat in food, imparting fatlike properties, including richness and providing a slippery "mouth feel" reducing the absorption of fat in products being fried.

Combinations:

Hundreds of US, EU and Japanese patents have been granted to various combinations of HPMC and other ingredients used as capsules. These include carrageen, potassium chloride, polyvinyl chloride, polyethylene glycol, ammonium ions, gelatin, catechin, mannan gums, locust bean gum, pectin, glycerin, acetic acid, calcium gluconate, sucrose fatty acid esters[155,156,157].

9.3 EXPERIMENT

9.3.1 Preparation of HPMC gels:

Preparation of HPMC gels

2g, 3g was dispersed in demineralised water 100ml by stirring at 800rpm for 60 minutes to get 2%, 3% HPMC gel. HPMC gels were centrifuged at 1,500-2,000 rpm before measurement in order to remove entrapped air. All gels were allowed to equilibrate for at least 16 hours at room temperature.

Incorporation of liposomes in to the gel

Liposomes containing zidovudine were mixed in to the 1%, 2%, 3% HPMC with electrical mixer (25 rpm, 3minutes). The concentration of incorporated drug in liposomes was equal to 10mg.

Table-70 : Percentage release of conventional liposomes and HPMC K4 100 gel 2%, 3%

S.No	Time in hours	% release from conventional liposomes	% release from HPMC 2%	% release from HPMC 3%
1	0	0	0	0
2	0.5	12.5	7.99	6.58
3	1	21.45	12.35	9.65
4	1.5	32.6	18.54	13.2
5	2	40.6	23.86	17.99
6	2.5	50.74	26.53	22.56
7	3	66.2	28.34	25.62
8	3.5	71.25	38.1	28.65
9	4	84.6	40.25	32.47
10	6	98.01	53.2	39.65
11	8	98.23	64	50.14
12	10	98.56	74	59.6
13	12	98.99	80	65.2
14	14	98.74	87.4	73.1
15	16	98.01	94.6	79.5
16	18	99.01	96	87.4
17	20	98.38	98.56	90.6
18	22	98.12	98.23	93.4
19	24	98.64	98.47	94.78

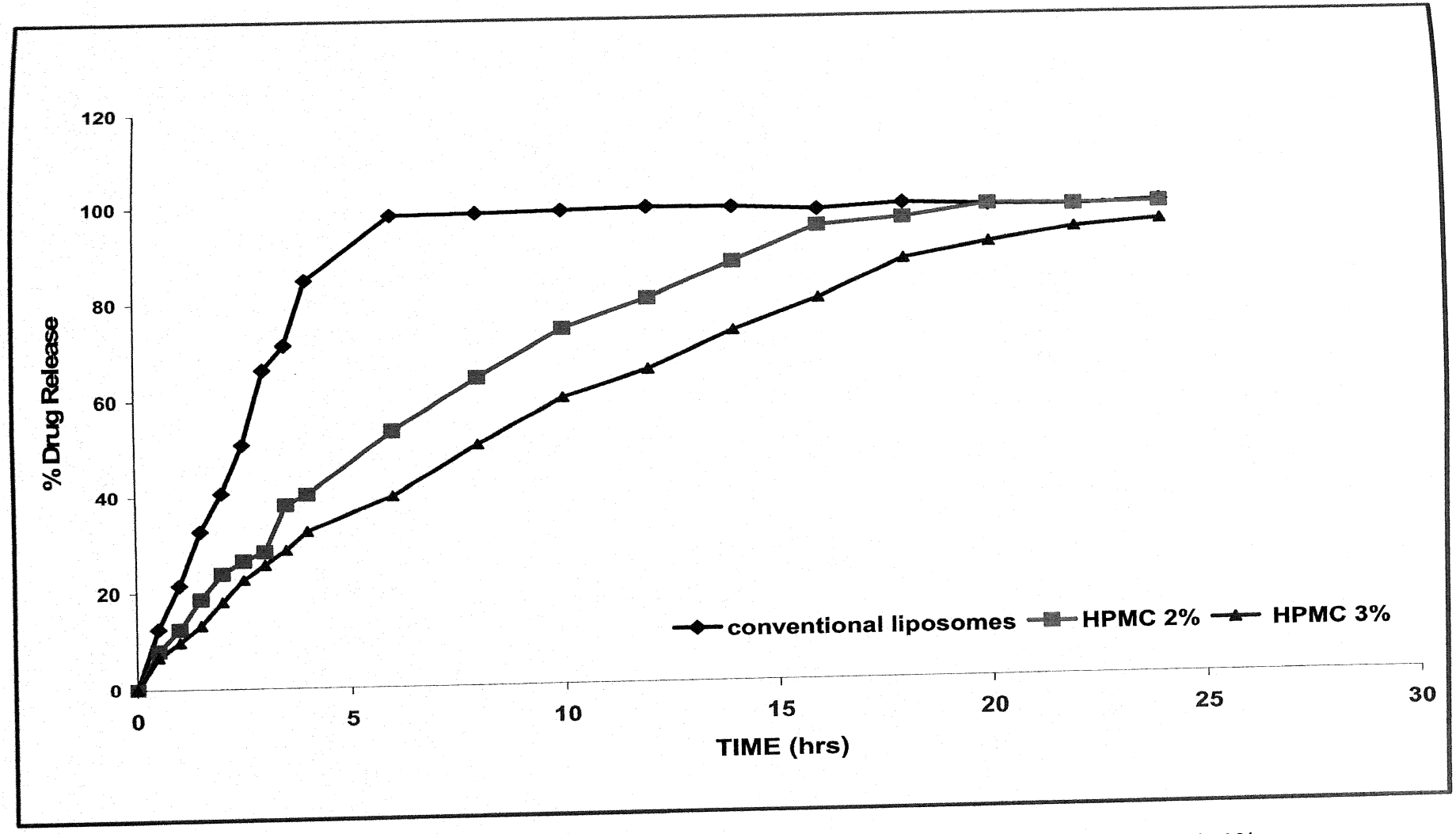


Figure-118: Percentage release of conventional liposomes and HPMC K4 100 gel 2%, 3%

Table-71: Percentage drug release model from HPMC K4 100

Model		Percentage release from HPMC K4 100	
		2%HPMC K4 100	3% HPMC K4 100
Zero Order	R	0.9275	0.9685
	k	3.0309	2.6667
	SSQ	1061	392
1st Order	R	0.9770	0.9944
	k	-0.0429	-0.0361
	SSQ	305	82
Matrix	R	0.9844	0.9760
	k	12.1973	10.5794
	SSQ	236	300
Peppas	R	0.9939	0.9975
	k	7.1962	5.5423
	SSQ	229	41
Hix Crow	R	0.9643	0.9885
	k	-0.0127	-0.0108
	SSQ	490	152
		PEPPAS	PEPPAS

Table-72: Average drug release from 2% HPMC K4 100

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	1061	305	236	229	490
1.	0.5	3.994	6.145	3.504	21.440	0.160	4.440
2.	1	6.250	10.363	4.202	35.370	0.895	6.247
3.	1.5	9.480	24.345	10.542	29.792	0.015	15.133
4.	2	12.319	39.157	16.773	24.308	0.286	24.137
5.	2.5	13.893	39.885	13.845	29.084	0.006	22.031
6.	3	15.071	35.743	8.956	36.665	0.429	16.834
7.	3.5	20.232	92.620	39.530	6.693	7.198	56.513
8.	4	21.697	91.650	35.114	7.278	5.753	52.697
9.	6	28.603	108.524	34.865	1.624	8.121	56.236
10.	8	34.494	105.012	29.586	0.000	8.360	50.132
11.	10	40.273	99.281	28.979	2.895	10.439	47.526
12.	12	43.906	56.780	13.408	2.732	3.002	23.993
13.	14	48.379	35.368	10.375	7.514	1.734	16.561
14.	16	52.846	18.941	10.098	16.458	1.194	12.606
15.	18	54.514	0.002	0.500	7.649	3.110	0.292
16.	20	56.739	15.039	0.751	4.802	15.369	2.614
17.	22	57.544	83.443	12.590	0.111	54.348	24.073
18.	24	58.647	198.640	31.871	1.227	108.494	58.078

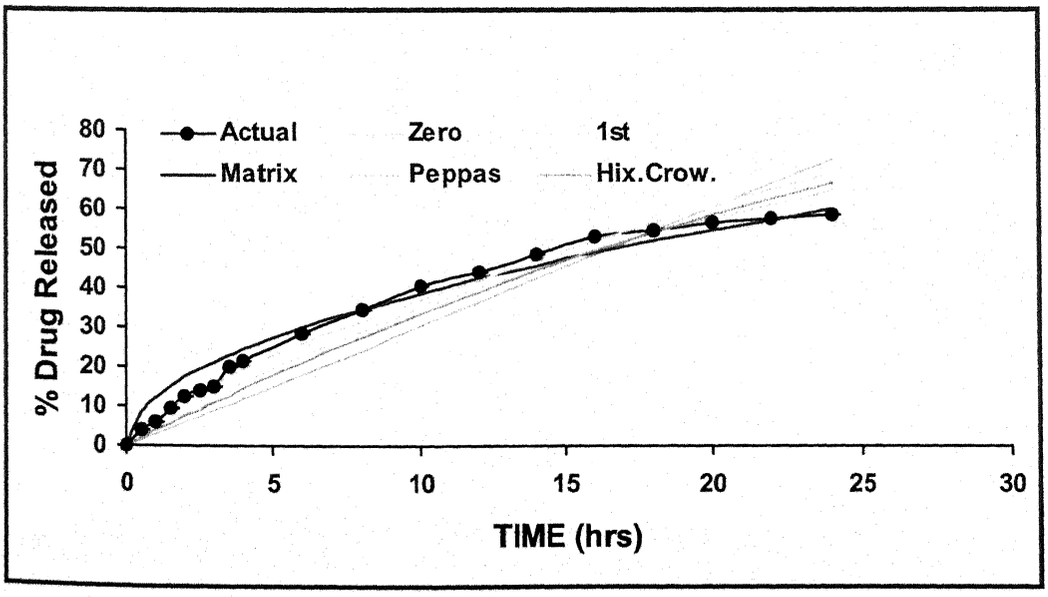


Figure-119: Average drug release from 2% HPMC K4 100.

Table-73: Average drug release from 3% HPMC K4 100.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	392	82	300	41	152
1.	0.5	3.300	3.867	2.280	17.479	0.000	2.836
2.	1	4.891	4.949	1.805	32.354	0.424	2.813
3.	1.5	6.794	7.807	2.310	37.980	0.518	3.997
4.	2	9.287	15.632	5.371	32.198	0.001	8.576
5.	2.5	11.743	25.768	9.661	24.844	0.515	14.730
6.	3	13.554	30.845	10.783	22.753	0.830	16.972
7.	3.5	15.276	35.316	11.560	20.393	1.172	18.746
8.	4	17.489	46.548	16.288	13.464	3.237	25.477
9.	6	21.407	29.233	3.692	20.313	0.018	9.930
10.	8	27.042	32.588	3.783	8.298	0.413	10.470
11.	10	32.281	31.510	3.853	1.379	1.138	10.115
12.	12	35.667	13.440	0.242	0.963	0.016	2.323
13.	14	40.273	8.636	0.337	0.474	0.008	1.776
14.	16	44.199	2.346	0.092	3.541	0.048	0.490
15.	18	49.004	1.007	1.436	16.974	0.230	1.391
16.	20	51.425	3.645	0.000	16.915	1.196	0.249
17.	22	53.716	24.519	1.236	16.766	7.278	4.355
18.	24	55.353	74.802	6.885	12.422	23.709	16.932

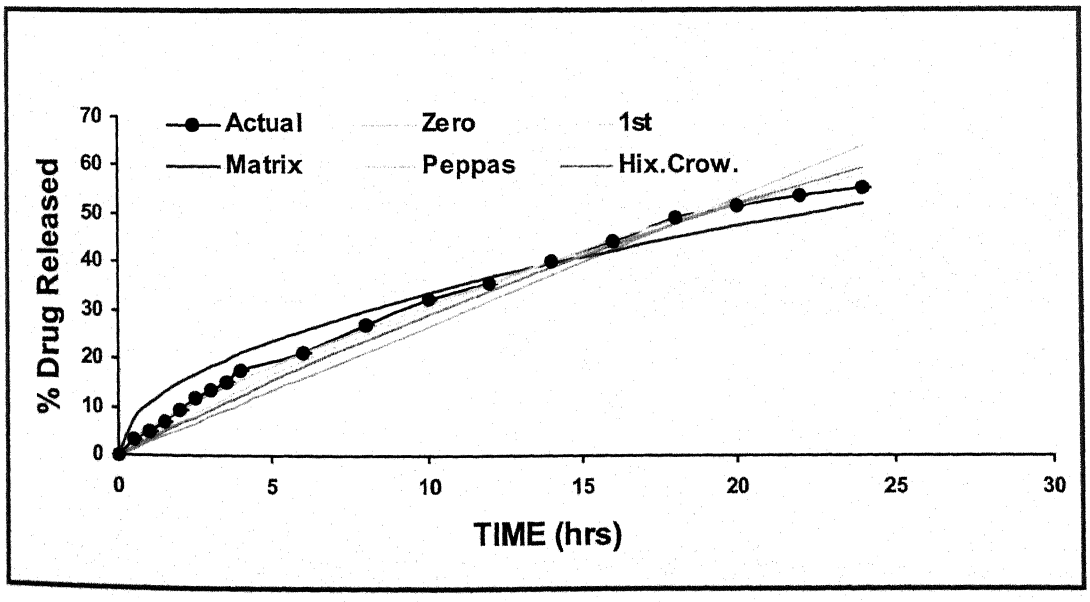


Figure-120: Average drug release from 3% HPMC K4 100.

Table-74: Percentage release of conventional liposomes and HPMC K4 15 gel 2%, 3%.

S.No	Time in hours	% release from conventional liposomes	% release from HPMC K4 15 2%	% release from HPMC K4 15 3%
1	0	0	0	0
2	0.5	12.5	7.99	5.3
3	1	21.45	11.6	7.1
4	1.5	32.6	22.2	11.6
5	2	40.6	23.1	14.2
6	2.5	50.74	28	18.7
7	3	66.2	31.1	23.1
8	3.5	71.25	37.3	24
9	4	84.6	40.25	29.3
10	6	98.01	49.8	36
11	8	98.23	59.6	45.3
12	10	98.56	74	59.6
13	12	98.99	79.1	62.2
14	14	98.74	86.2	69.3
15	16	98.01	90.7	76
16	18	99.01	90.7	81.8
17	20	98.38	95.1	83.6
18	22	98.12	94.2	88
19	24	98.64	94.2	88.9

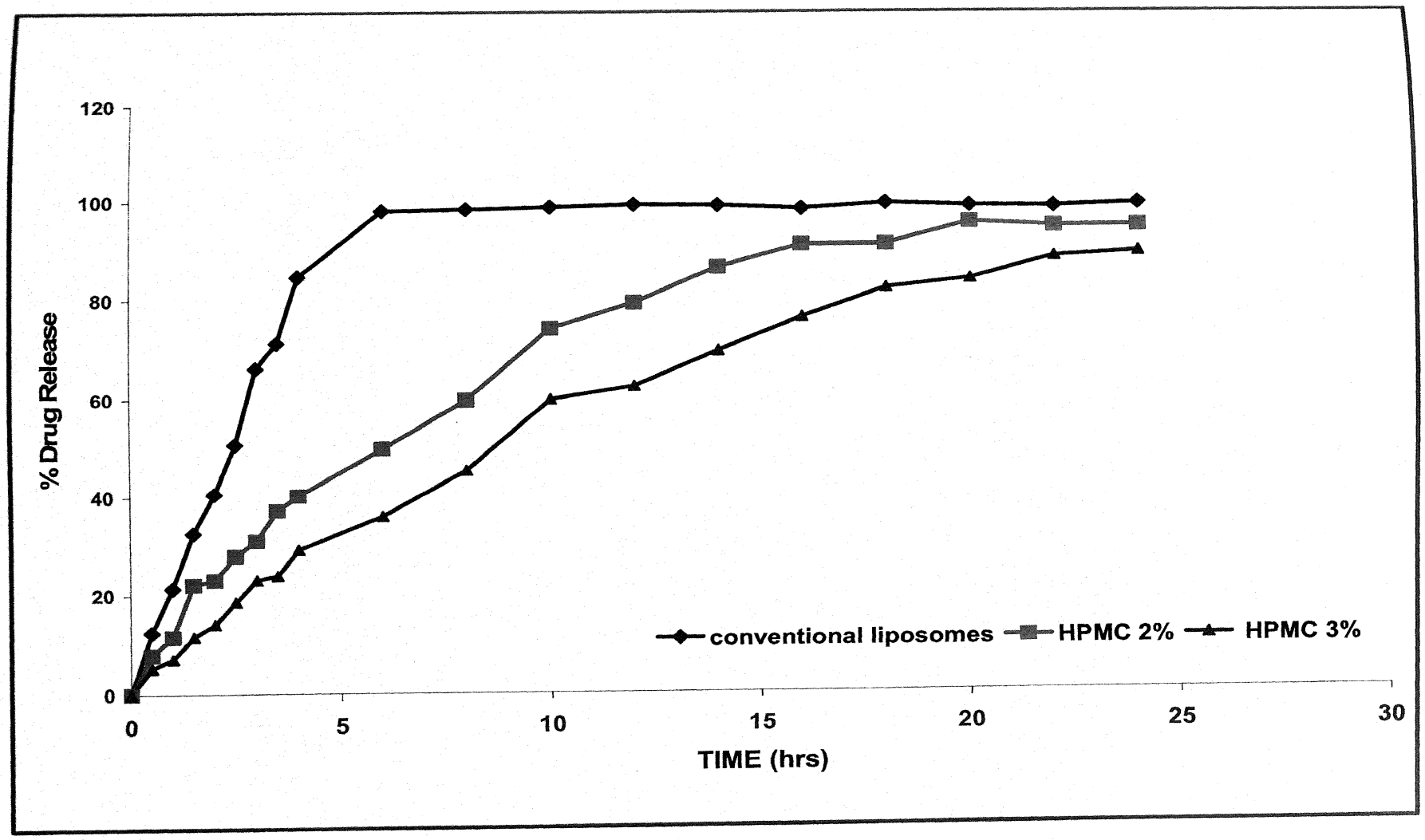


Figure-121: Percentage release of conventional liposomes and HPMC K4 15 gel 2%, 3%.

Table-75: Percentage drug release model from HPMC K4 15

Model		Percentage release from HPMC K4 15	
		2%HPMC K4 15	3% HPMC K4 15
Zero Order	R	0.9165	0.9733
	k	2.9404	2.4960
	SSQ	1112	305
1st Order	R	0.9697	0.9942
	k	-0.0410	-0.0330
	SSQ	379	62
Matrix	R	0.9866	0.9697
	k	11.8711	9.8651
	SSQ	185	346
Peppas	R	0.9913	0.9959
	k	7.4094	4.4679
	SSQ	220	78
Hix Crow	R	0.9558	0.9892
	k	-0.0122	-0.0100
	SSQ	564	117
		PEPPAS	PEPPAS

Table-76: Average drug release from 2%HPMC K4 15

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	1112	379	185	220	564
1.	0.5	3.996	6.382	3.880	19.340	0.351	4.769
2.	1	5.889	8.692	3.521	35.788	2.312	5.224
3.	1.5	11.304	47.513	28.579	10.468	2.244	35.186
4.	2	11.927	36.558	16.513	23.630	0.001	23.121
5.	2.5	14.706	54.101	24.755	16.511	0.561	34.367
6.	3	16.493	58.853	24.329	16.552	0.437	35.343
7.	3.5	19.895	92.228	42.801	5.353	5.215	58.749
8.	4	21.753	99.833	44.149	3.955	5.951	61.810
9.	6	26.973	87.061	26.924	4.430	1.996	44.177
10.	8	32.309	77.194	19.136	1.605	1.269	34.534
11.	10	40.151	115.489	42.889	6.819	14.201	62.916
12.	12	43.421	66.186	21.136	5.281	4.633	32.802
13.	14	47.744	43.276	16.897	11.069	3.375	23.812
14.	16	50.874	14.651	7.886	11.494	0.280	9.814
15.	18	52.785	0.020	0.403	5.860	3.348	0.205
16.	20	54.897	15.300	1.032	3.269	14.771	3.063
17.	22	55.407	86.171	15.786	0.075	53.775	27.946
18.	24	56.349	202.248	38.752	3.266	105.666	66.095

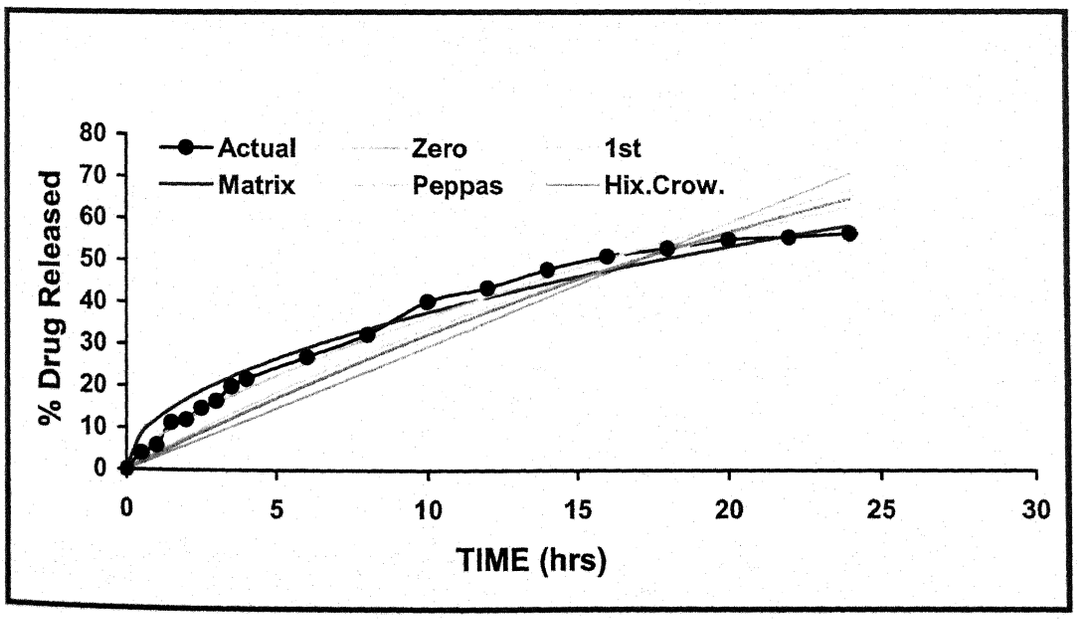


Figure-122: Average drug release from 2% HPMC K4 15.

Table-77: Average drug release from 3% HPMC K4 15.

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	305	62	346	78	117
1.	0.5	2.652	1.970	1.033	18.697	0.010	1.351
2.	1	3.606	1.232	0.131	39.178	0.743	0.413
3.	1.5	5.933	4.791	1.227	37.814	0.070	2.280
4.	2	7.354	5.578	0.945	43.529	0.214	2.208
5.	2.5	9.724	12.138	3.282	34.507	0.134	5.885
6.	3	12.126	21.516	7.336	24.605	1.654	11.648
7.	3.5	12.851	16.937	3.812	31.411	0.331	7.489
8.	4	15.682	32.463	11.060	16.392	4.036	17.473
9.	6	19.427	19.809	2.183	22.448	0.214	6.355
10.	8	24.352	19.226	1.363	12.604	0.189	5.140
11.	10	31.943	48.765	14.867	0.558	10.939	23.988
12.	12	33.863	15.302	1.409	0.096	0.476	4.300
13.	14	38.018	9.454	1.093	1.224	0.204	2.870
14.	16	42.103	4.699	1.229	6.985	0.070	2.103
15.	18	45.714	0.619	0.910	14.901	0.088	0.871
16.	20	47.445	6.121	0.706	11.070	7.009	1.608
17.	22	50.539	19.113	1.095	18.215	12.654	3.666
18.	24	51.802	65.629	8.255	12.062	38.796	17.694

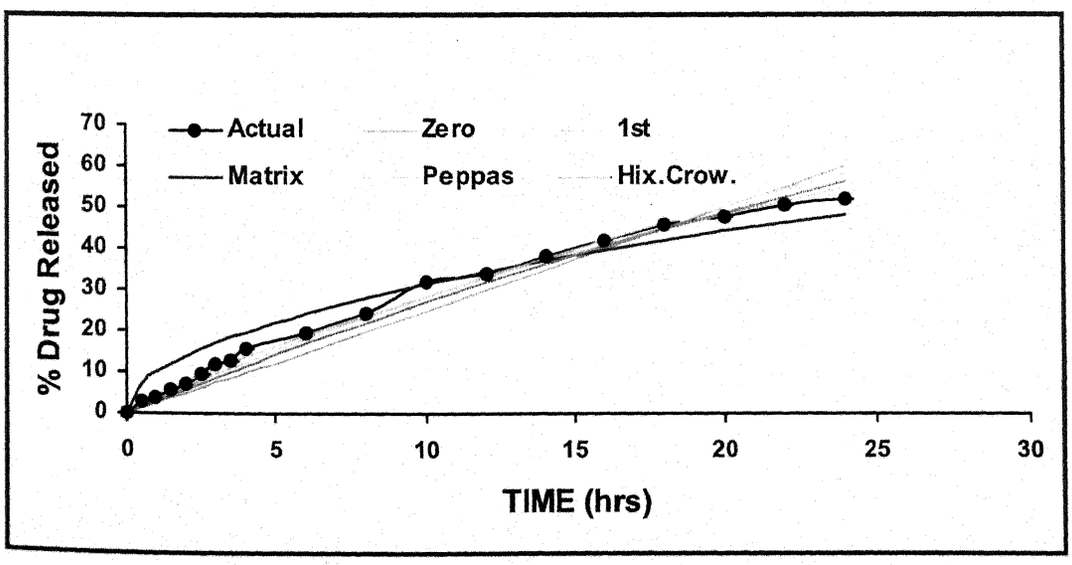


Figure-123: Average drug release from 3% HPMC K4 15.

Table-78: Stability of 2% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	99.12	95.3	94.4
3.	2	94	92.4	88.4
4.	3	90.4	84.8	75.2

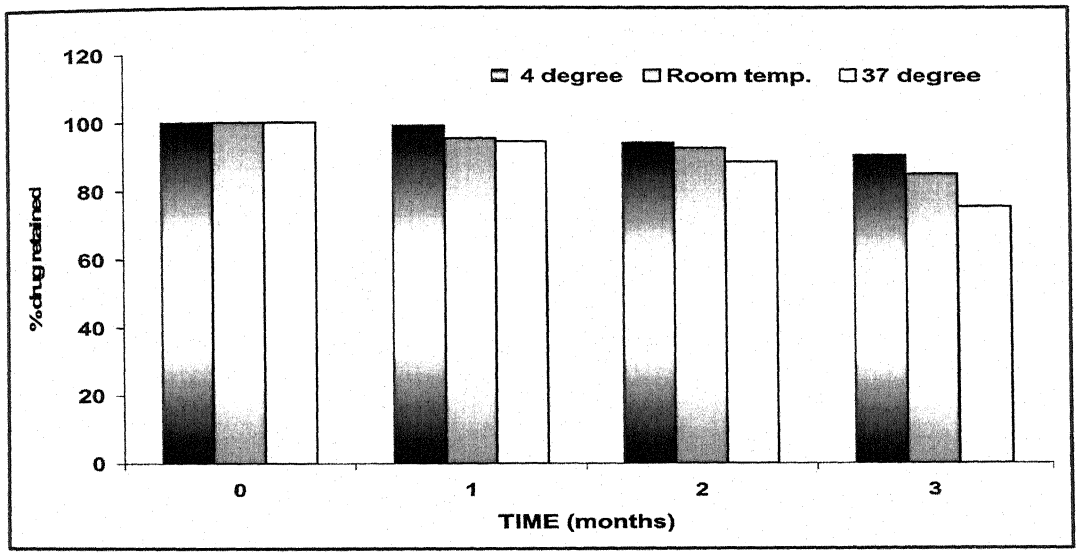


Figure-124: Stability of 2% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

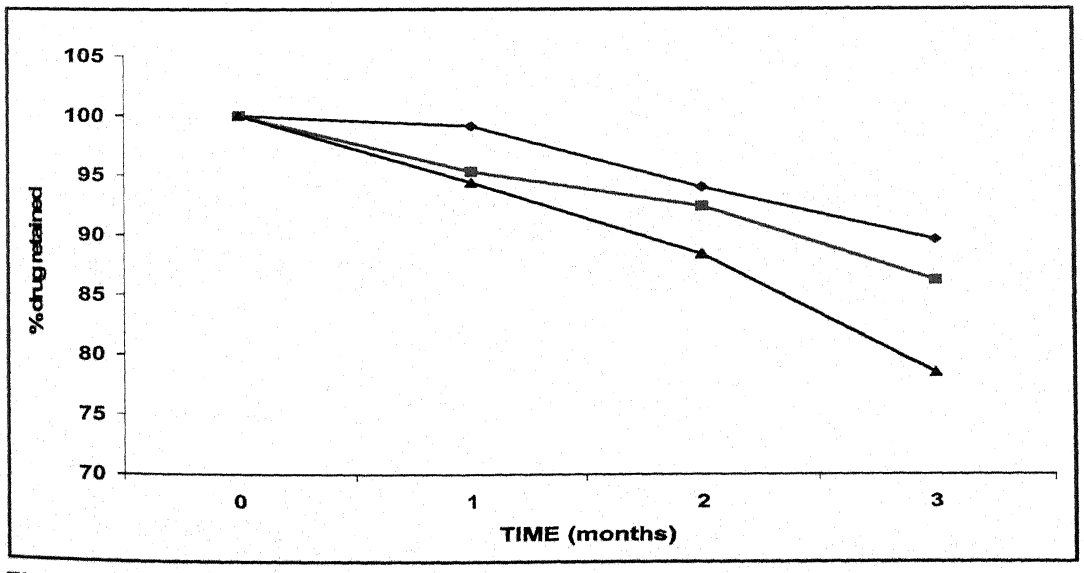


Figure-125: Stability of 2% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

Table-79: Stability of 3% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	96.24	95.01	94.48
3.	2	93.01	90.41	86.32
4.	3	89.6	86.2	78.5

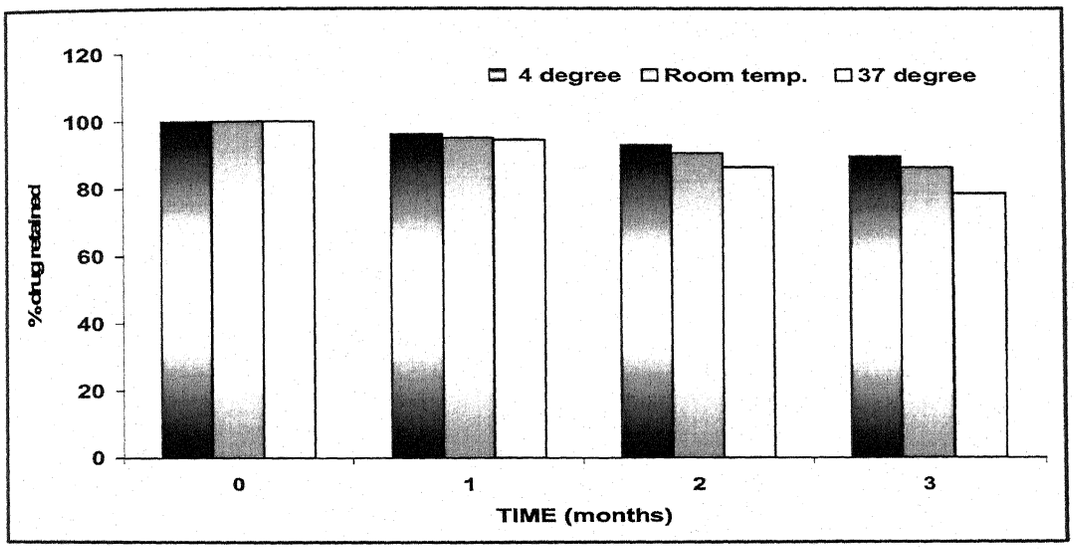


Figure-126: Stability of 3% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

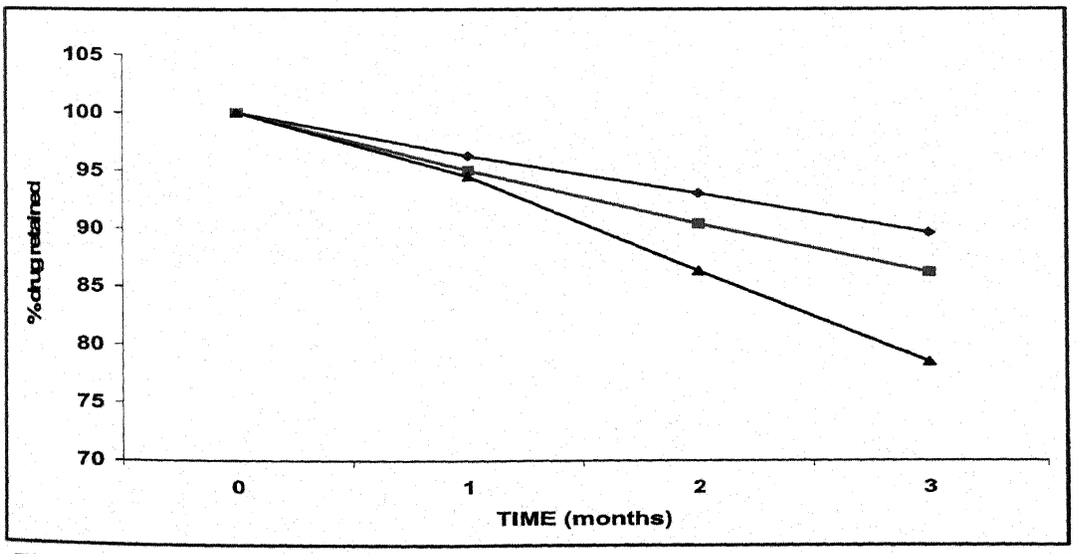


Figure-127: Stability of 3% HPMC K4 100 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

Table-80: Stability of 2% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	97	94	94
3.	2	95	88.8	86
4.	3	93.5	82	79

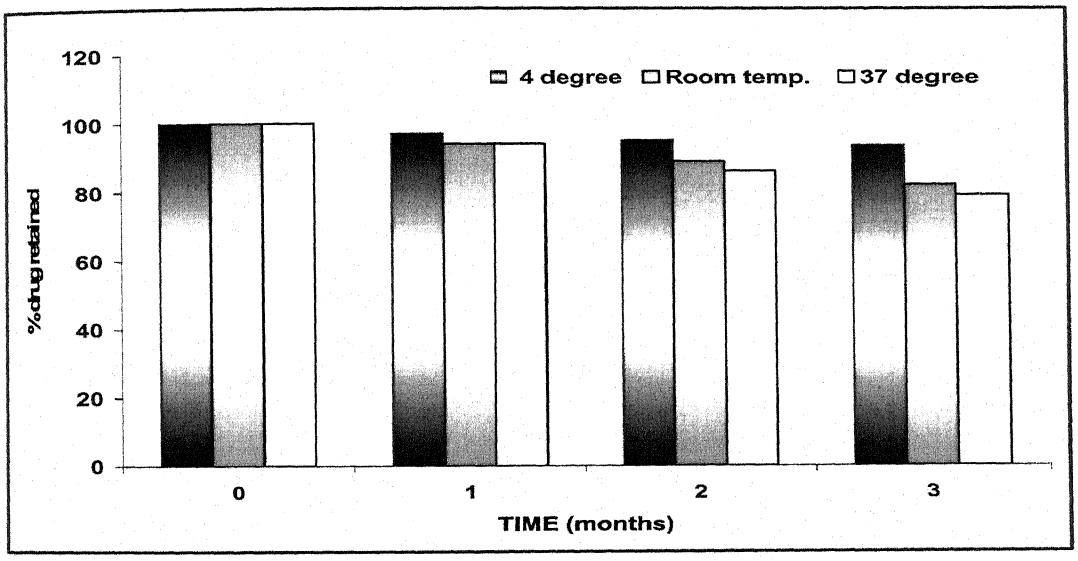


Figure-128: Stability of 2% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

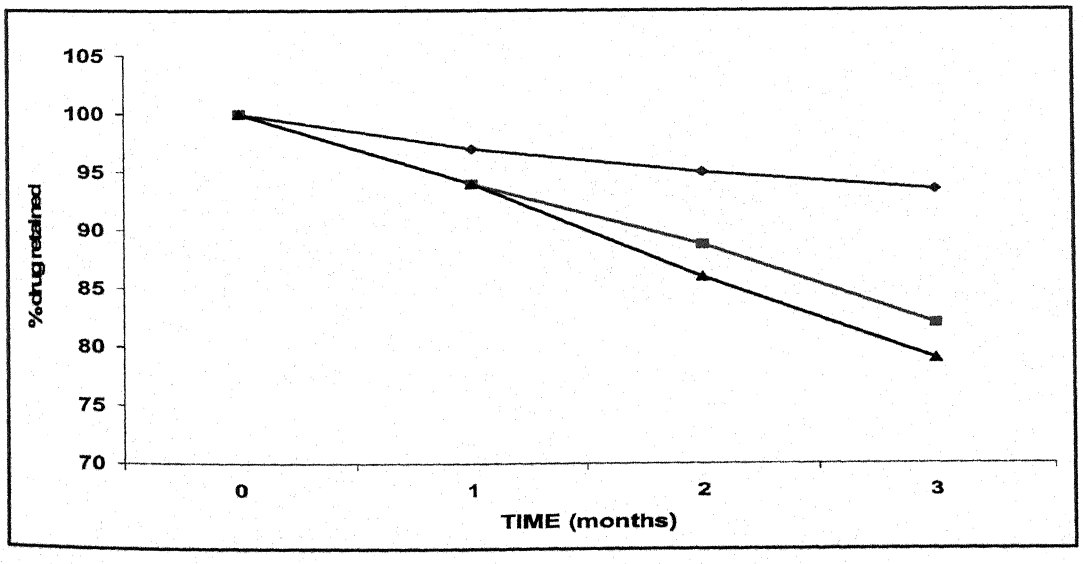


Figure-129: Stability of 2% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

Table-81: Stability of 3% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	97.5	97.8	95
3.	2	96.6	93.5	84.25
4.	3	93.4	89	80

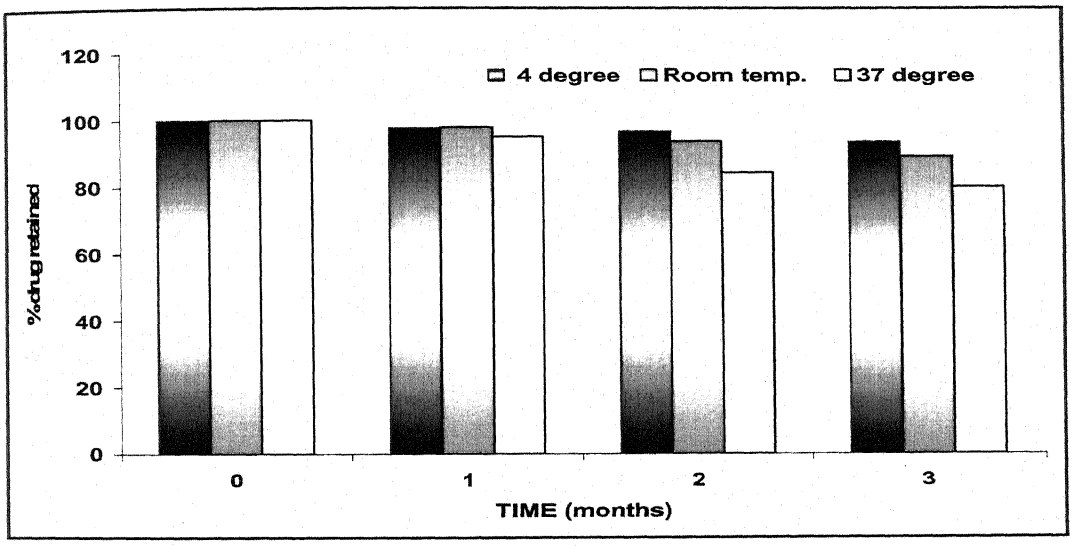


Figure-130: Stability of 3% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

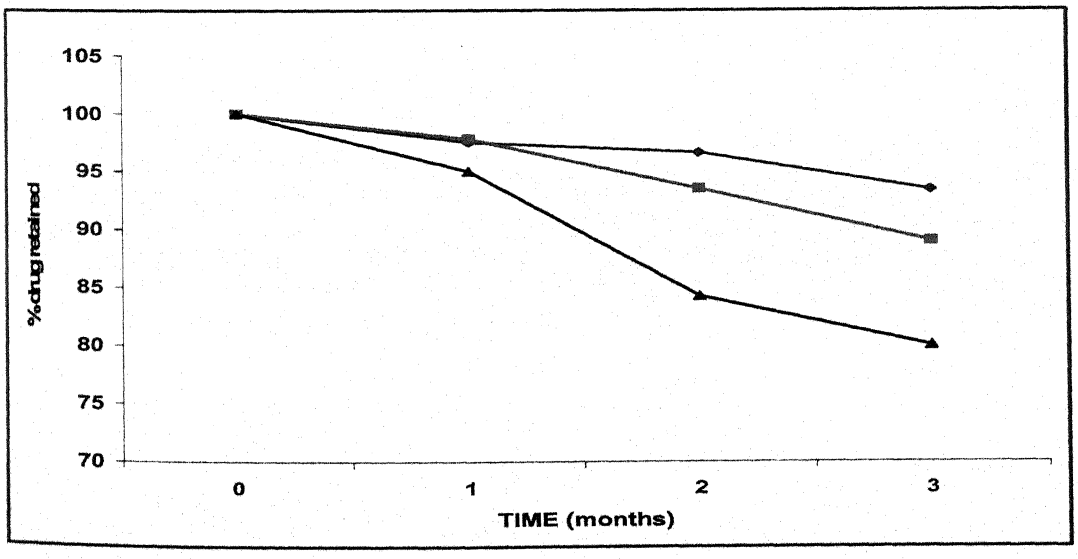


Figure-131: Stability of 3% HPMC K4 15 liposomal gel containing zidovudine at 4^0C, room temperature and 37^0C after three months.

Chapter- 10

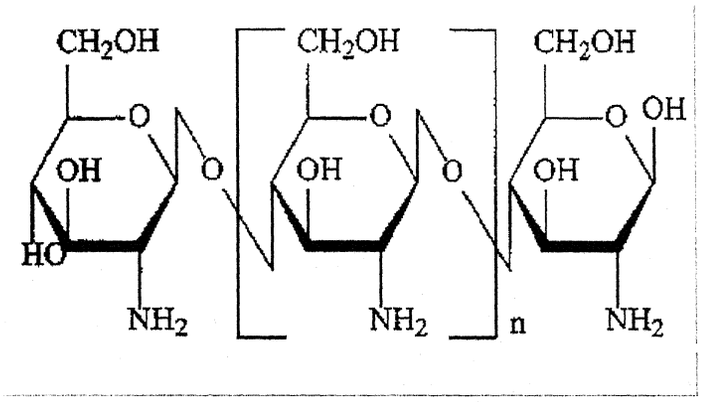
Chitosan

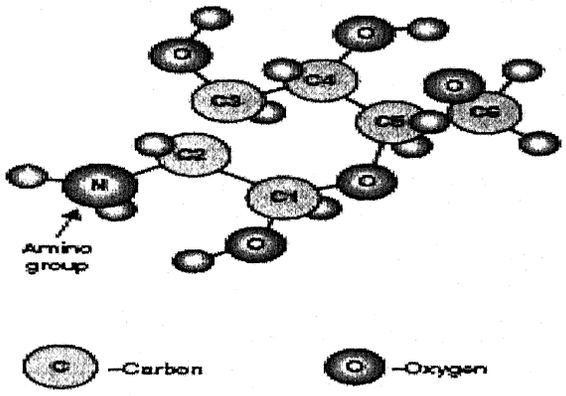
CHITOSAN

10.1 INTRODUCTION

Chitosan is a copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-Dglucopyranose.This polycationic biopolymer is generally obtained by alkaline deacetylation from chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps[158].

Structure of Chitosan.





The main parameters influencing the characteristics of chitosan are its molecular weight (MW) and degree of deacetylation (DD), representing the proportion of deacetylated units. These parameters are determined by the conditions set during preparation. Moreover, they can be further modified. For example, the DD can be lowered by reacetylation[159] and MW can be lowered by acidic depolymerisation[160]. Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications.

The main reasons for this increasing attention are certainly its interesting intrinsic properties. Indeed, chitosan is known for being biocompatible allowing its use in various medical applications such as topical ocular application[161], implantation[162]. Moreover, chitosan is metabolised by certain human enzymes, especially lysozyme, and is considered as biodegradable[163]. In addition, it has been reported that chitosan acts as a penetration enhancer by opening epithelial tight-junctions[164]. Due to its positive charges at physiological pH, chitosan is also bioadhesive, which increases retention at the site of application[165]. Chitosan also promotes wound-healing and has bacteriostatic effects[166]. Finally, chitosan is very abundant, and its production is of low cost and ecologically interesting. In medical and pharmaceutical applications, chitosan is used as a component in hydrogels.. There are several possible definitions of a hydrogel; we will use the one given by Peppas who defined hydrogels as macromolecular networks swollen in water or biological fluids[167,168]. Examples of networks related to hydrogels that correspond to this definition will also be introduced. Due to the various possible definitions of a hydrogel, different methods of classification are possible. Based on the definition given here, hydrogels are often divided into three classes depending on the nature of their network, namely entangled networks, covalently crosslinked networks and networks formed by secondary interactions.

The latter class contains all the intermediary cases situated between the two other classes representing the extreme[169]. However, with respect to chitosan hydrogels, this classification is not entirely suitable. Certainly, there are no strict borders between these classes, but there is a continuum of various gels ranging from entangled chitosan hydrogels to covalently crosslinked chitosan hydrogels. Therefore, the following modified classification for chitosan hydrogels, i.e. the separation of chemical and physical hydrogels. Chemical hydrogels are formed by irreversible covalent links, as in covalently crosslinked chitosan hydrogels. Physical hydrogels are formed by various reversible links. These can be ionic interactions as in ionically crosslinked hydrogels and polyelectrolyte complexes (PEC), or secondary interactions as in chitosan/poly(vinyl alcohol) (PVA) complexed hydrogels, grafted

chitosan hydrogels and entangled hydrogels. The latter are formed by solubilisation of chitosan in an acidic aqueous medium which is the simplest way to prepare a chitosan hydrogel[170]. Entangled chitosan hydrogels will not be discussed further in this study, as they are limited by their lack of mechanical strength and their tendency to dissolve. Moreover, they do not exhibit characteristics that allow drug delivery to be efficiently controlled—such as the modification of their properties in response to changes in their physicochemical environment, such as pH or temperature.

The present study is exclusively concerned with chitosan hydrogels formed by the addition of a crosslinker, namely covalently or ionically crosslinked hydrogels. A second study entitled 'Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications' will discuss hydrogels formed by direct interaction between polymeric chains, without the addition of crosslinkers. They can be formed by complexation with another polymer, generally ionic, or by aggregation after chitosan grafting. In cross-linked hydrogels, polymeric chains are interconnected by crosslinkers, leading to the formation of a 3D network. Crosslinkers are molecules of MW much smaller than the MW of the chains between two consecutive crosslinks.

The properties of cross linked hydrogels depend mainly on their crosslinking density, namely the ratio of moles of cross linking agent to the moles of polymer repeating units. Moreover, a critical number of crosslinks per chain is required to allow the formation of a network, such as that of a hydrogel. Depending on the nature of the cross linker, the main interactions forming the network are covalent or ionic bonds. The structures and interactions forming the covalently and ionically cross linked hydrogels will be presented, their principles of formation and properties will be considered and examples of medical or pharmaceutical applications will be given. Their potential biocompatibility will be discussed, although some examples will refer to systems that are still in development, while others have already been tested in animals. Consequently, their potential biocompatibility will sometimes be evaluated based on the intrinsic biocompatibility of their components[171].

10.2 EXPERIMENTAL

10.2.1Preparation of Chitosan gels

The structured vehicle of chitosan (1%, 2%, 3%) was prepared by continuous mixing of chitosan with a 1% (w/v) solution of Lactic acid for 2 hours.

10.2.2 Incorporation of liposomes in to the gel

Liposomes containing zidovudine were mixed in to the 1%, 2%, 3% Chitosan with electrical mixer (25 rpm, 3minutes). The concentration of incorporated drug in liposomes was equal to 10mg.

Table-82: Percentage release of zidovudine from 1%, 2%, 3% Chitosan gels.

S. No	Time in hours	1%Chitosan gel	2%Chitosan gel	3%Chitosan gel
1.	1	10.18	9.24	5.5
2.	2	22.46	13.1	7.3
3.	4	30.45	18.5	11
4.	6	36.24	23.4	14
5.	8	41.2	29.5	21.3
6.	10	46	31.6	23.4
7.	12	48.8	37.1	28
8.	14	58	41.2	31.6
9.	16	61.1	45.3	36
10.	18	63.2	47.4	37.1
11.	20	66.6	56	38
12.	22	70.8	59.1	43.3
13.	24	73.5	61.1	46.7

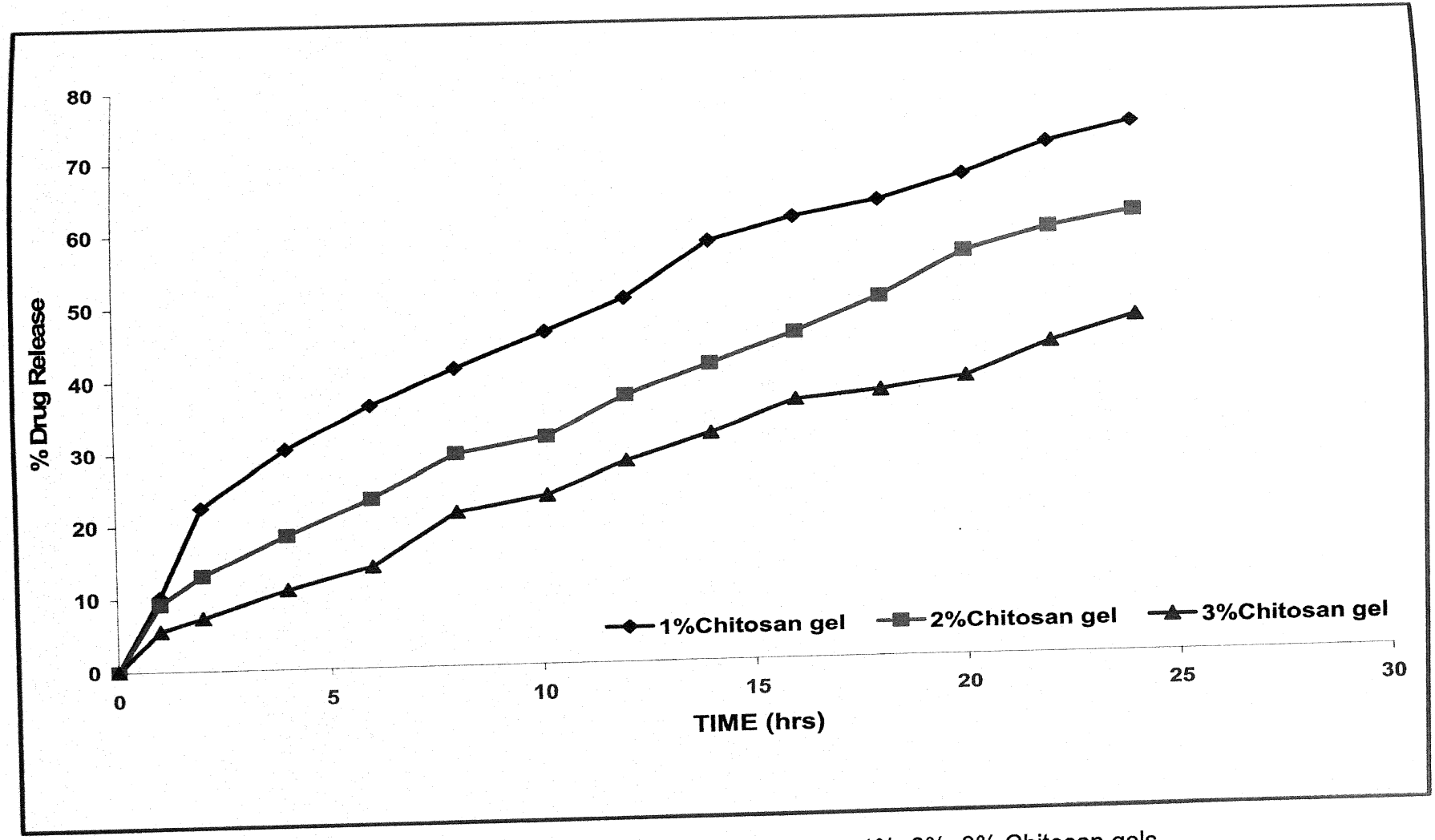


Figure- 132 : Percentage release of zidovudine from 1%, 2%, 3% Chitosan gels

Table-83: Percentage release from 1%, 2%,3% Chitosan Gel

Model		Percentage release from Chitosan 1%		
		Chitosan 1%	Chitosan 2%	Chitosan 3%
Zero Order	R	0.9336	0.7332	0.9900
	k	1.9980	1.4728	1.1411
	SSQ	290	710	19
1st Order	R	0.9711	0.7841	0.9951
	k	-0.0247	-0.0173	-0.0128
	SSQ	149	685	09
Matrix	R	0.9930	0.7997	0.9597
	k	8.2330	6.0771	4.6063
	SSQ	31	554	74
Peppas	R	0.9902	0.5728	0.9920
	k	6.1303	7.0010	2.3007
	SSQ	12	638	09
Hix Crow	R	0.9607	0.7689	0.9938
	k	-0.0077	-0.0055	-0.0041
	SSQ	188	693	12
		MATRIX	MATRIX	ISTORDER

Table-84: Percentage release from 1% Chitosan Gel

RESULTS			Zero	1st order	Matrix	Peppas	Hix.Crow.
Sr.No.	Time	Avg. %R	290	149	31	12	188
1	0	0.000	0.000	0.000	0.000	-	0.000
2	1	5.090	9.562	7.035	9.877	1.082	7.910
3	2	11.330	53.783	42.425	0.098	3.880	46.361
4	4	15.548	57.097	37.795	0.842	1.580	44.157
5	6	18.749	45.709	24.849	2.009	0.196	31.353
6	8	21.594	31.471	13.518	2.863	0.051	18.769
7	10	24.415	19.664	6.471	2.625	0.349	10.064
8	12	26.270	5.260	0.404	5.063	2.824	1.385
9	14	31.364	11.504	4.617	0.313	0.430	6.494
10	16	33.741	3.141	1.245	0.654	0.179	1.762
11	18	35.154	0.658	0.513	0.050	0.420	0.539
12	20	37.488	6.115	2.163	0.447	0.479	3.073
13	22	40.241	13.805	2.746	2.641	0.051	4.989
14	24	42.314	31.797	5.678	3.924	0.131	10.747

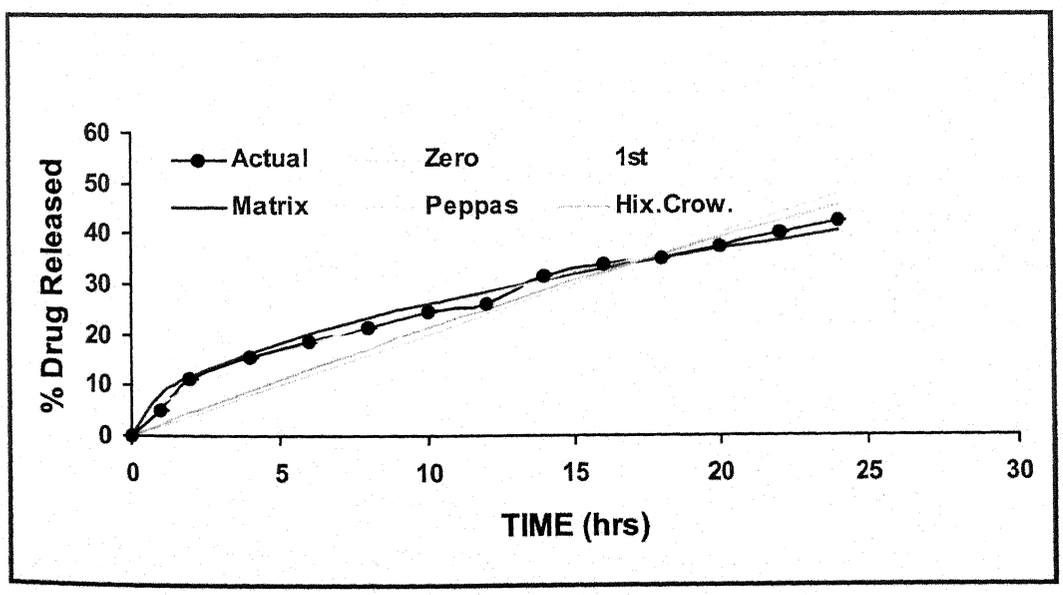


Figure-133: Percentage release from 1% Chitosan Gel

Table-85: Percentage release from 2% Chitosan Gel

RESULTS			Zero	1st order	Matrix	Peppas	Hix. Crow.
Sr. No.	Time	Avg. %R	710	685	554	638	693
1	1	4.625	9.934	8.450	2.110	5.647	8.967
2	2	16.649	187.793	175.386	64.883	54.566	179.741
3	4	19.680	190.135	168.587	56.638	55.140	175.981
4	6	11.976	9.859	4.422	8.464	6.042	6.055
5	8	15.793	16.084	8.118	1.949	0.177	10.527
6	10	17.152	5.876	1.545	4.268	0.348	2.689
7	12	20.218	6.475	2.088	0.695	1.256	3.276
8	14	3.906	279.301	310.919	354.648	269.531	300.354
9	16	24.701	1.294	0.240	0.155	10.574	0.492
10	18	26.218	0.085	0.330	0.189	13.865	0.230
11	20	30.988	2.349	2.895	14.516	56.479	2.746
12	22	33.093	0.480	1.956	21.060	75.687	1.425
13	24	34.668	0.460	0.418	23.973	88.397	0.069

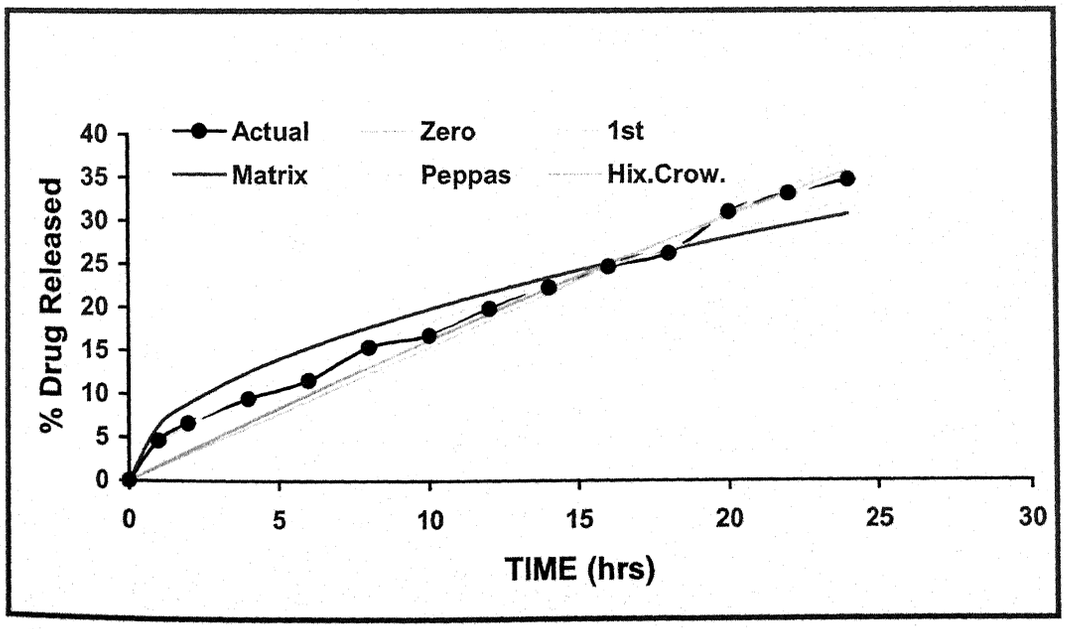


Figure-134: Percentage release from 2% Chitosan Gel

Table-86: Percentage release from 3% Chitosan Gel

RESULTS			Zero	1st order	Matrix	Peppas	Hix.Crow.
Sr. No.	Time	Avg. %R	19	9	74	9	12
1	1	2.758	2.615	2.207	3.416	0.209	2.346
2	2	3.706	2.028	1.388	7.885	0.024	1.597
3	4	5.656	1.192	0.440	12.650	0.673	0.657
4	6	7.252	0.164	0.021	16.252	2.293	0.002
5	8	11.025	3.596	1.660	4.015	0.025	2.231
6	10	12.294	0.779	0.076	5.167	0.295	0.228
7	12	14.844	1.325	0.361	1.239	0.018	0.613
8	14	16.910	0.875	0.249	0.106	0.167	0.413
9	16	19.434	1.386	0.827	1.018	1.446	0.995
10	18	20.336	0.041	0.062	0.629	0.184	0.054
11	20	21.159	2.763	2.056	0.312	0.143	2.258
12	22	24.219	0.783	0.110	6.828	1.195	0.250
13	24	26.358	1.055	0.010	14.380	2.826	0.144

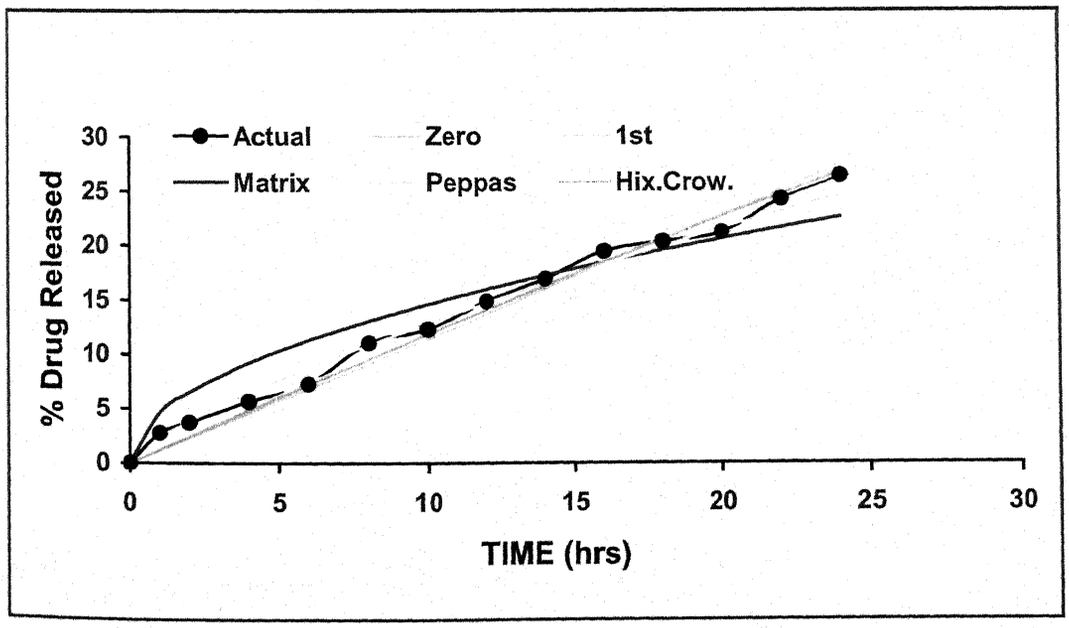


Figure-135 : Percentage release from 3% Chitosan Gel

Table-87: Stability of 1% chitosan liposomal gel at 4^0C, room temp. and 37^0C

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	97.6	94	93.8
3.	2	94.8	89.01	81.1
4.	3	92.5	87	85

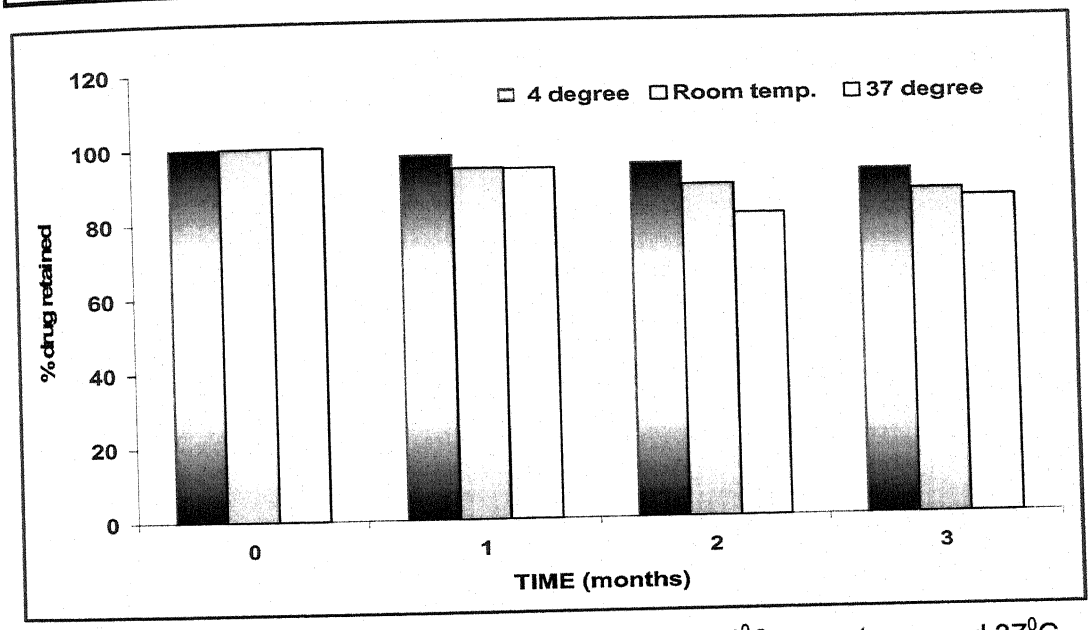


Figure-136: Stability of 1% chitosan liposomal gel at 4^0C, room temp. and 37^0C

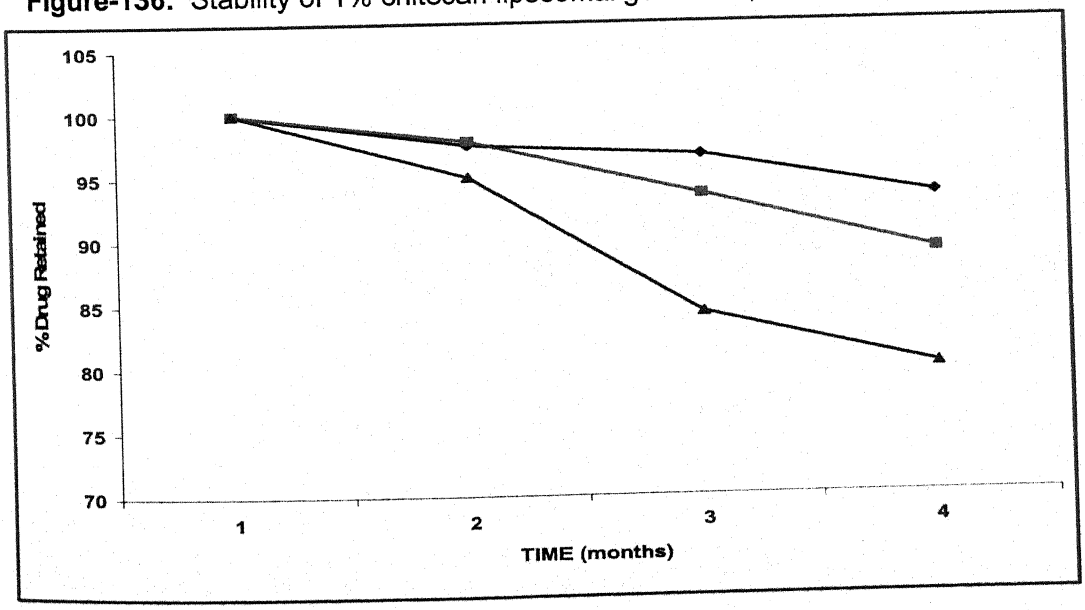


Figure-137: Stability of 1% chitosan liposomal gel at 4^0C, room temp. and 37^0C

Table-88: Stability of 2% chitosan liposomal gel at 4^0C, room temp. and 37^0C

S. No.	Time in months	% drug retained at 4^0C	% drug retained at Room temp.	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	98	96	97.1
3.	2	96	93.	92.08
4.	3	94.0	88.0	90.1

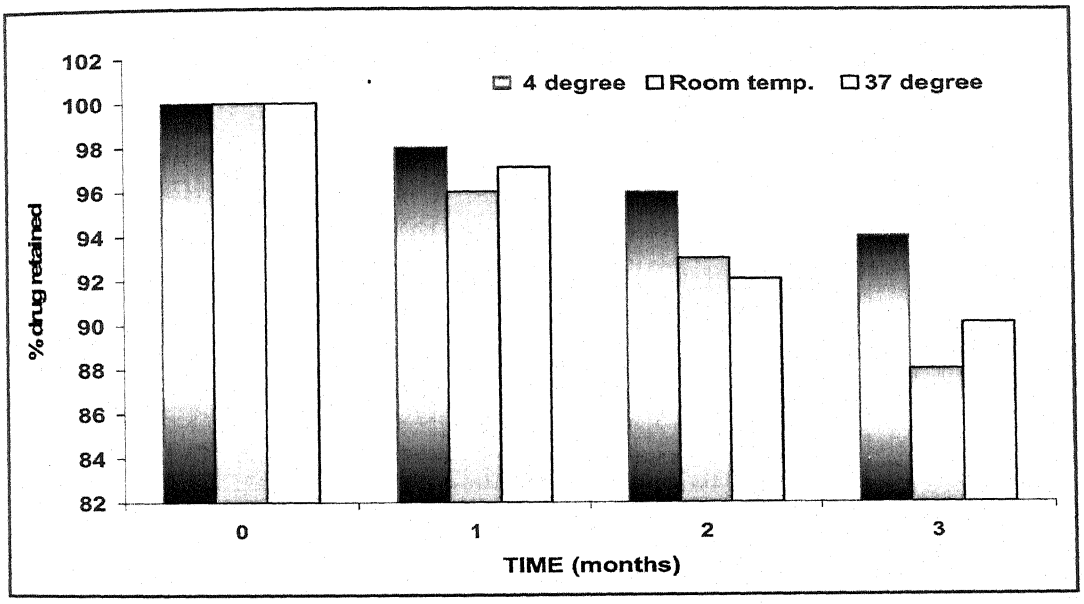


Figure-138: Stability of 2% chitosan liposomal gel at 4^0C, room temp. and 37^0C

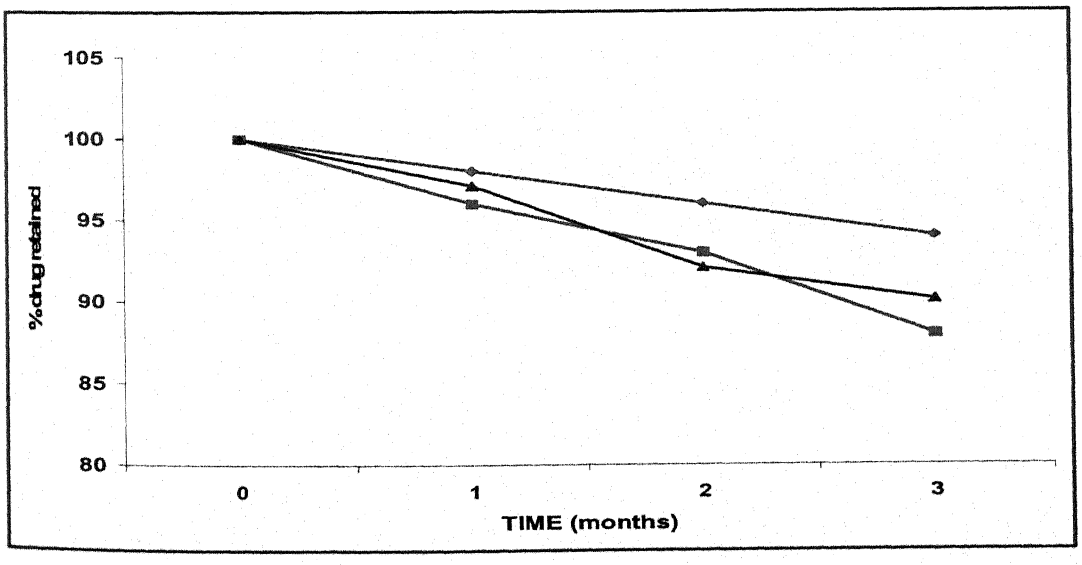


Figure-139: Stability of 2% chitosan liposomal gel at 4^0C, room temp. and 37^0C

Table-89: Stability of 3% chitosan liposomal gel at 4^0C, room temp. and 37^0C

S. No.	Time in months	% drug retained at 4^0C	% drug retained at room temp.	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	99.01	98.12	97.00
3.	2	96.8	94.00	94.04
4.	3	94.5	89.4	90.4

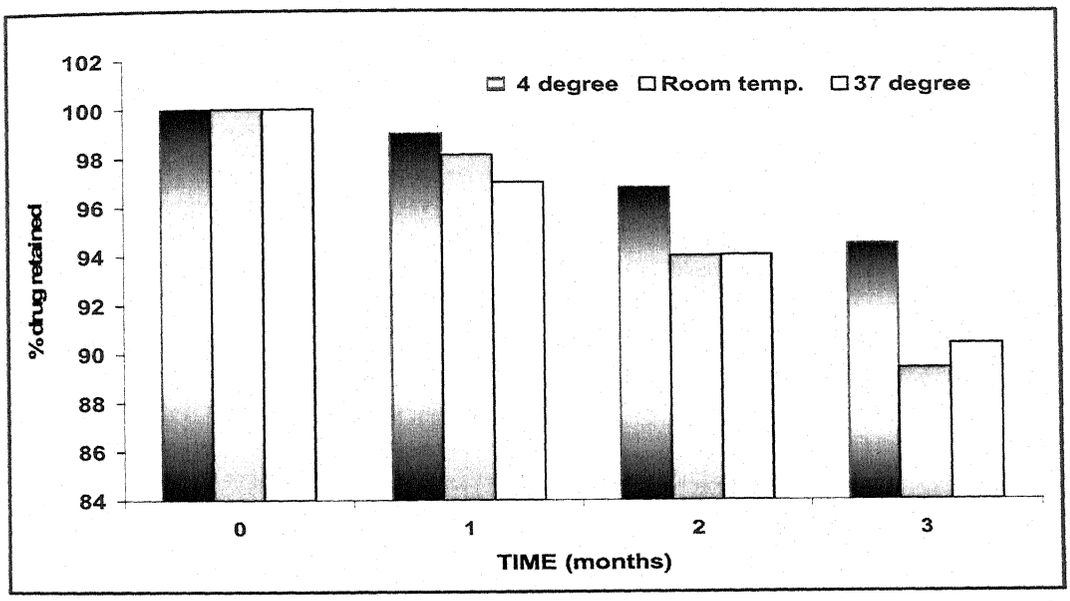


Figure-140: Stability of 3% chitosan liposomal gel at 4^0C, room temp. and 37^0C

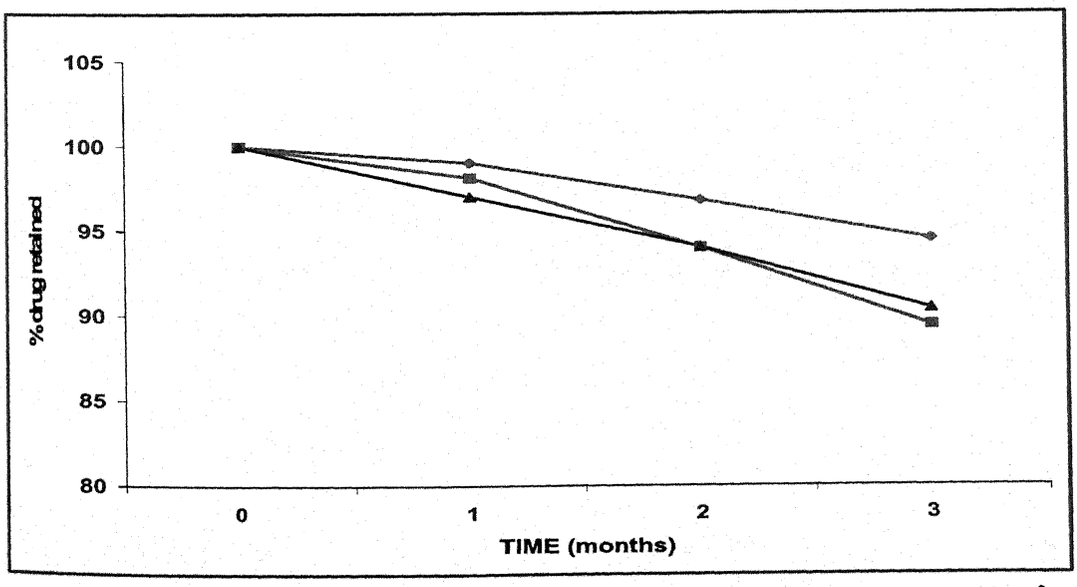


Figure-141: Stability of 3% chitosan liposomal gel at 4^0C, room temp. and 37^0C

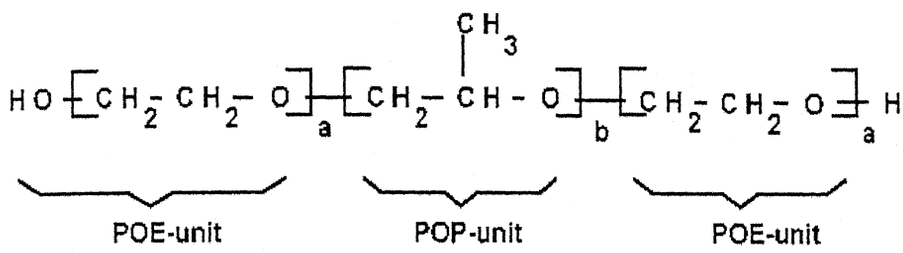
Chapter- 11

Polaxamer

POLAXAMER

11.1 INTRODUCTION

Poloxamer block copolymers have been introduced in the late 1950s and since then they have been proposed for diverse pharmaceutical applications[172,173].



Polaxamer-188(a= Ca. 79 and b= Ca.28).

Poloxamer Characteristics:

The character of poloxamer in terms of molecular weight, appearance, hydrophilicity, solubility and it is determined by the chain length of the polyoxypropylene(EO-)units and polyoxypropylene(PO-) units.

Molecular weight: 8600

Molecular number: 7600

In general Poloxamer seemed to interact strongly with both negatively (phosphatidylglycerol-containing), positively (stearylamine-containing) and neutral liposomes, as shown by the shielding of the surface charge. This was confirmed by increase in the size of the liposomes, which were particularly marked for positively charged liposomes. In the case of neutral liposomes, no change in zeta potential was observed, but size measurements showed that a layer of gel was adsorbed. The thickness of this layer was lowest for liposomes containing a PEG-substituted lipid, which, as explained above, would be expected to prevent adsorption. These results indicate the feasibility of incorporating different types of liposome into poloxamer gels.

Poloxamer copolymer (ethylene oxide and propylene oxide blocks) shows thermo reversible properties, which is of the almost interest in optimizing drug formulation (fluid state at room temperature facilitating administration and gel state above sol-gel transition temperature at body temperature promoting prolonged release of pharmacological agents). Pharmaceutical evaluation consists in determining the rheological behavior (flow curve or oscillatory studies), sol-gel transition temperature, in vitro drug release using either synthetic or physiological membrane or bioadhesion characteristics. Poloxamer formulations led to enhanced solubilisation of poorly water-soluble drugs and prolonged release profile for many applications (e.g., oral, rectal, topical, ophthalmic, nasal and injectable preparations). Poloxamer actions by optimizing sol-gel transition temperature or increasing bioadhesive properties. Inclusion of liposomes or micro or nanoparticles in Poloxamer formulations offers interesting prospects, as well. Besides these promising data, Poloxamer has been held responsible for lipidic profile alteration and possible renal toxicity, which compromises its development for parenteral applications.

Poloxamer promotes stabilization of included drugs. Interesting findings on interactions between Poloxamer and liposomes have been documented, as well. Poloxamer is either co-solubilised with lipid during preparation of liposomes or added afterwards to the already formed liposomes. Including Poloxamer in liposomal delivery system sterically stabilized liposomes and, hence, prolonged their half-life[174]. Poloxamer prevented the steric aggregation and the fusion of thawed egg phosphatidylcholine multilamellar vesicles[175]. Poloxamer 188 causes significant size-reduction of the multilamellar vesicles using quasi-elastic light scattering.

Poloxamer gel absorbs sweat gland secretion[176]. Gels and ointments containing non-steroidal anti-inflammatory drug (e.g., flurbiprofen, indomethacin, ketoprofen, piroxicam) have been formulated with Poloxamer . Most of the time, these preparations included a thickening agent like carbopol or cellulose derivatives[177,178]. Despite solubilisation enhancement of Poloxamer by means of micelle formation, a nonaqueous solvent like ethanol may be added to formulate a topical preparation with insoluble drugs like ketoprofen. In order to yield an increase

in the transdermal permeation, ketoprofen and piroxicam preparations have been formulated with Poloxamer and enhancers, which produced an increase in the anti-inflammatory activity with animal models (i.e., carragenin-induced rat foot swelling). Including absorption promoters in Poloxamer gel led to increase the permeation of drugs like ketoprofen. Four terpenes significantly increased ketoprofen in vitro diffusion through hairless mouse skins[179].

The bioadhesive characteristics are of great importance when prolonged residence-time is required, in particular with topical formulations (e.g., rectal, cutaneous or ophthalmic preparations). Bioadhesive force generally increases with gel strength and its value is modified by the same parameters (i.e., temperature and Poloxamer concentration). The presence of various solvents or ionic agents may alter the adhesion characteristics of poloxamer formulations as it has been previously noted. Because of its adhesion-promoting action, NaCl has been included in some Poloxamer gels to prolong residence-time in the site of administration[180]. The Zeta potential and size measurements confirm the adsorption of poloxamer onto the liposomes. The challenge for designing dosage forms is to retain the drug as long as possible in the applied area to allow time for sufficient absorption. Liposomes dispersed within a gel, which become solid at physiological temperature, might be one solution.

11.2 Preparation of poloxamer gel: 30g ,40g of poloxamer was dispersed in 100ml of water to get 30%,40% Poloxamer gel.

11.3 Percentage drug retained in the poloxamer gel.

The liposomes containing drug was separated from the Polaxamer gel by subjecting the gel to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai, India) at15, 000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained. The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer).

Table-89 : Percentage release of drug from 30%, 40% Poloxamer.

S. No	Time in hours	30% Poloxamer	40% Poloxamer
1	1	20	13.4
2	2	27.6	22.4
3	4	38.1	30.4
4	6	44	35.1
5	8	48	39.6
6	10	55.2	44.8
7	12	60	50.7
8	14	66.4	57.5
9	16	75.4	64.9
10	18	83.6	70.9
11	20	88	75.4
12	22	91	81.5
13	24	97	89.6

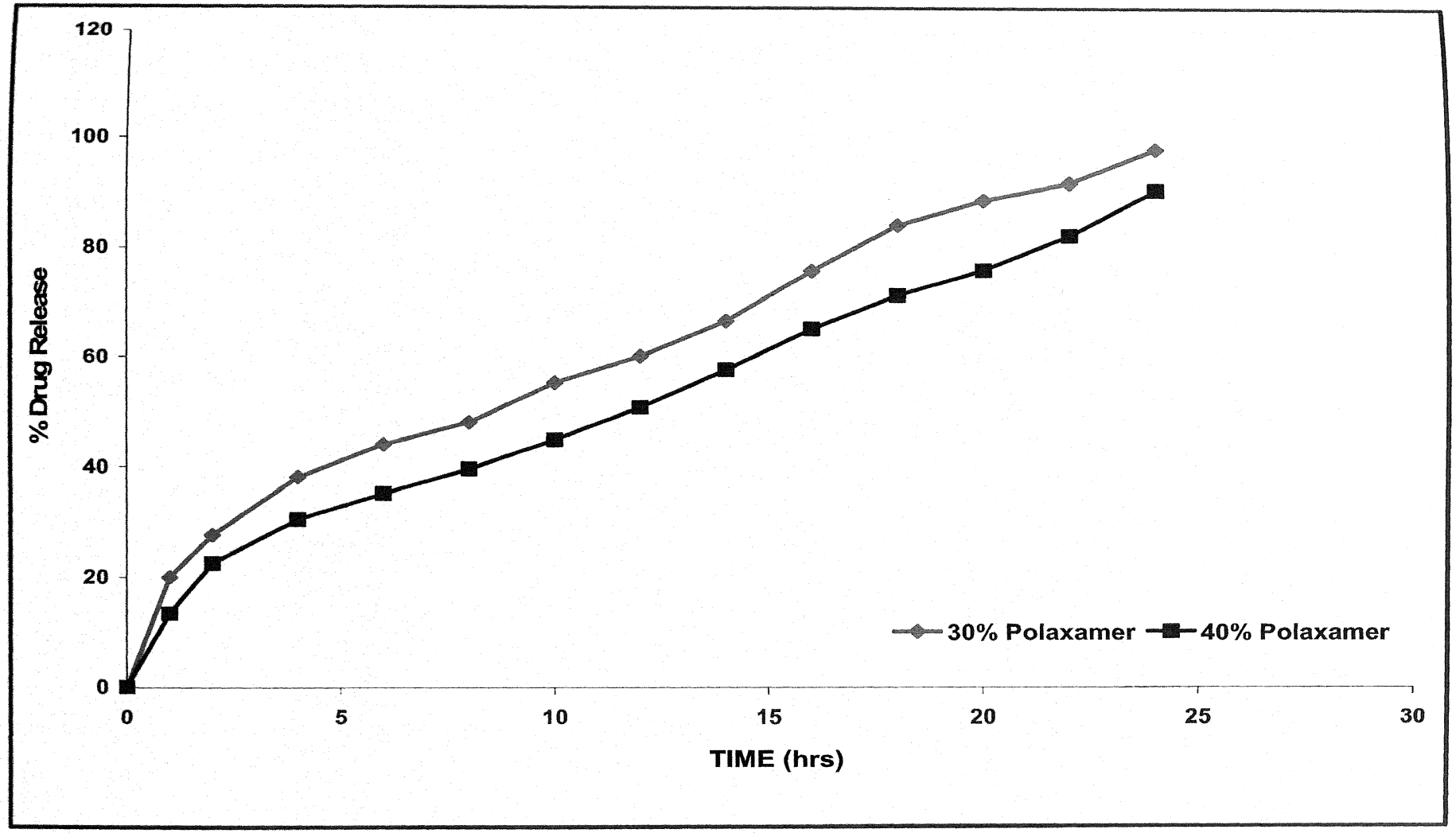


Figure-142: Percentage release of drug from 30%, 40% Poloxamer.

Table-90 : Percentage drug release from Poloxamer 30% &40%

Model	Percentage release from Poloxamer 30%& 40%		
		Poloxamer 30%	Poloxamer 40%
Zero Order	R	0.9466	0.9714
	k	2.5331	2.1921
	SSQ	378	170
1st Order	R	0.9849	0.9890
	k	-0.0339	-0.0281
	SSQ	175	92
Matrix	R	0.9864	0.9743
	k	10.3965	8.9283
	SSQ	99	153
Peppas	R	0.9913	0.9911
	k	9.3281	6.6338
	SSQ	76	71
Hix Crow	R	0.9767	0.9861
	k	-0.0102	-0.0086
	SSQ	222	108
		PEPPAS	PEPPAS

Table-91: Percentage release from Poloxamer 30%

RESULTS			Zero	1st order	Matrix	Peppas	Hix.Crow.
Sr. No.	Time	Avg. %R	378	175	99	76	222
1	1	10.044	56.410	44.991	0.124	0.512	49.146
2	2	14.008	79.954	55.455	0.483	0.254	64.052
3	4	19.533	88.363	46.797	1.588	0.000	60.350
4	6	22.864	58.759	19.749	6.770	1.986	31.146
5	8	25.331	25.670	2.430	16.600	8.824	7.640
6	10	29.385	16.431	0.373	12.193	6.236	3.109
7	12	32.345	3.795	1.214	13.463	7.812	0.011
8	14	36.257	0.630	2.423	6.984	3.597	0.645
9	16	41.339	0.655	0.308	0.061	0.135	0.013
10	18	46.169	0.328	0.214	4.244	6.440	0.279
11	20	49.193	2.160	0.006	7.280	9.229	0.184
12	22	51.578	17.226	1.039	7.920	9.088	3.332
13	24	55.499	28.046	0.043	20.855	21.407	2.226

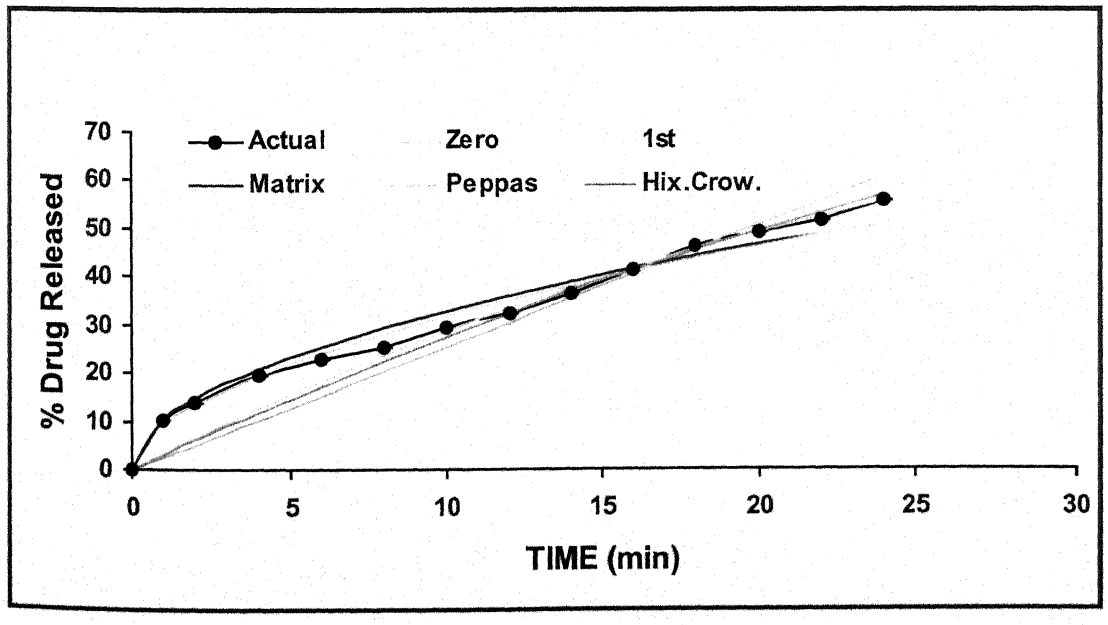


Figure-143: Percentage release from Poloxamer 30%

Table-92: Percentage release from Poloxamer 40%

RESULTS			Zero	1st order	Matrix	Peppas	Hix.Crow.
Sr.No.	Time	Avg. %R	170	92	153	71	108
1	1	6.706	20.375	15.494	4.939	0.005	17.226
2	2	11.382	48.972	35.039	1.549	1.717	39.878
3	4	15.561	46.142	24.344	5.270	0.073	31.369
4	6	18.213	25.613	7.312	13.369	1.712	12.469
5	8	20.822	10.796	0.487	19.631	5.729	2.516
6	10	23.818	3.598	0.449	19.502	7.499	0.037
7	12	27.216	0.831	1.951	13.781	5.870	0.404
8	14	31.115	0.182	1.953	5.250	1.982	0.648
9	16	35.384	0.096	0.666	0.109	0.018	0.202
10	18	39.043	0.172	0.412	1.353	1.449	0.301
11	20	42.040	3.245	0.883	4.458	2.963	1.349
12	22	45.806	5.857	0.083	15.430	9.636	0.737
13	24	50.675	3.744	2.676	48.099	32.212	0.478

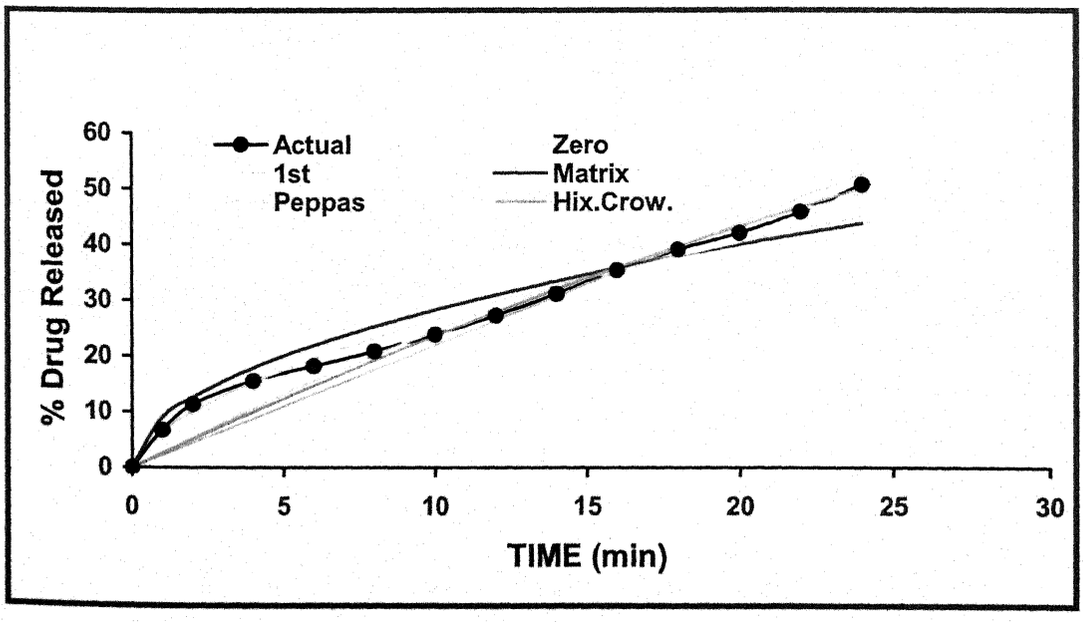


Figure-144: Percentage release from Poloxamer 40%

Table 93: Stability of 30% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	96	96	94.8
3.	2	91	89.1	88.1
4.	3	85	83	82.1

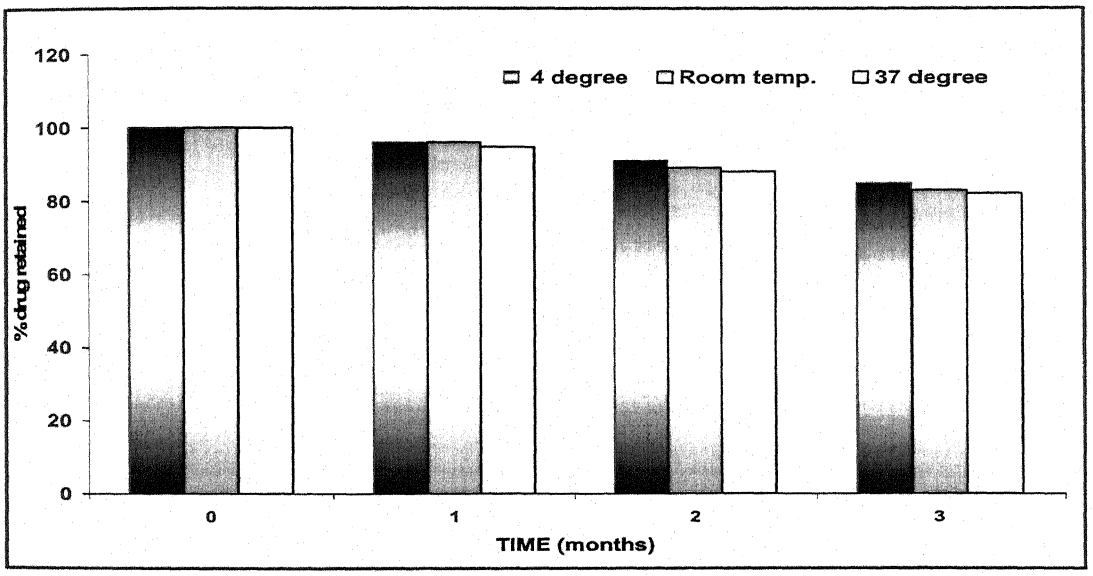


Figure-145(a): Stability of 30% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

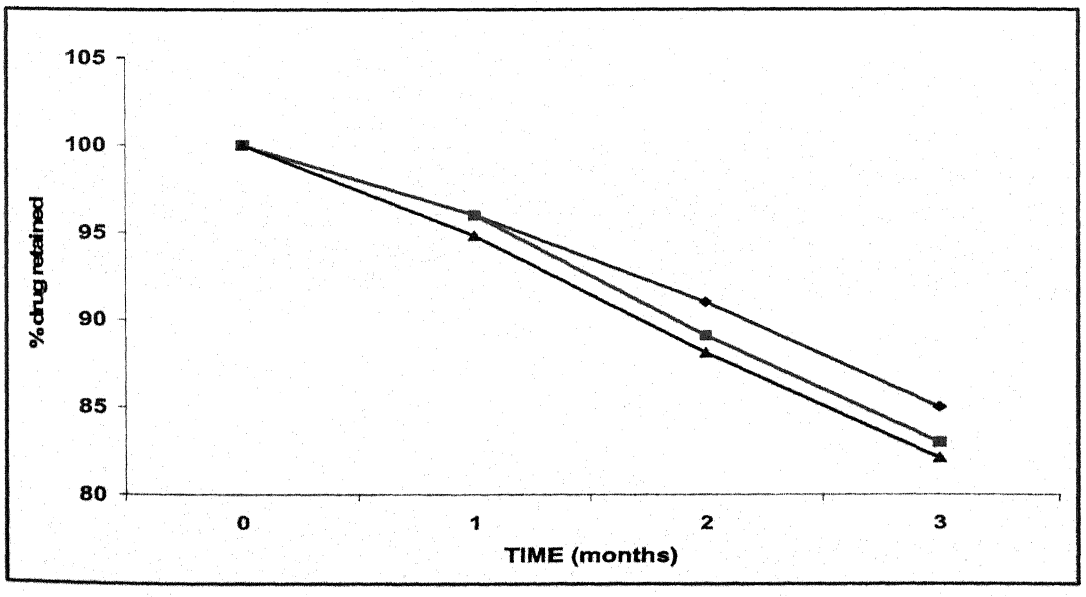


Figure-145(b): Stability of 30% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

Table 94: Stability of 40% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

S. No.	Time in months	% drug retained at 4^0C	% drug retained at RT	% drug retained at 37^0C
1.	Initial drug concentration	100	100	100
2.	1	95.5	94.6	94.5
3.	2	88.8	88.1	87
4.	3	82.2	81.83	80.06

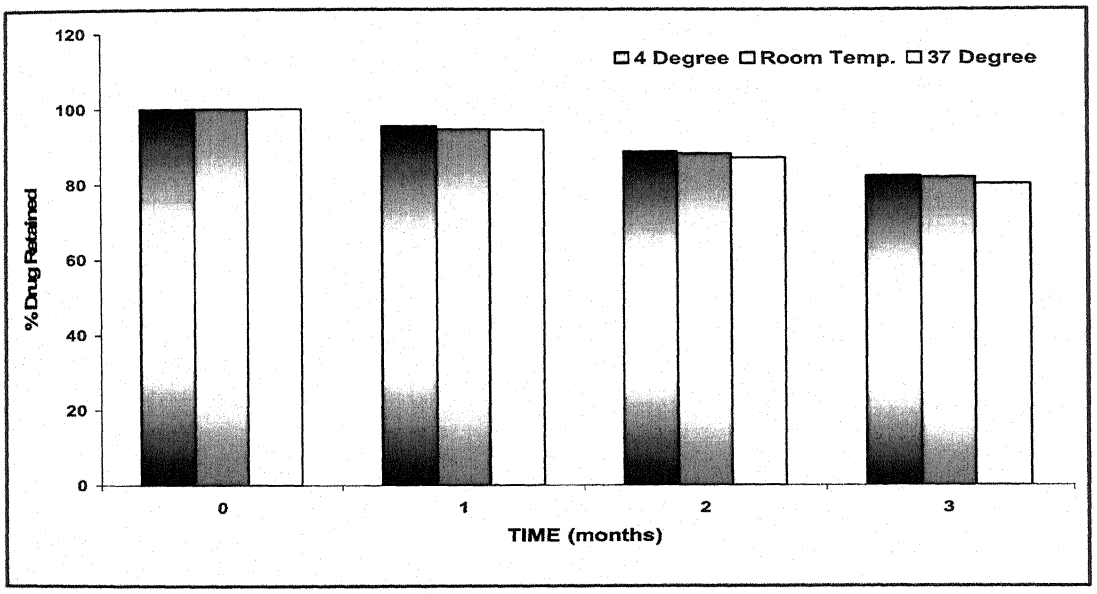


Figure-146(a): Stability of 40% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

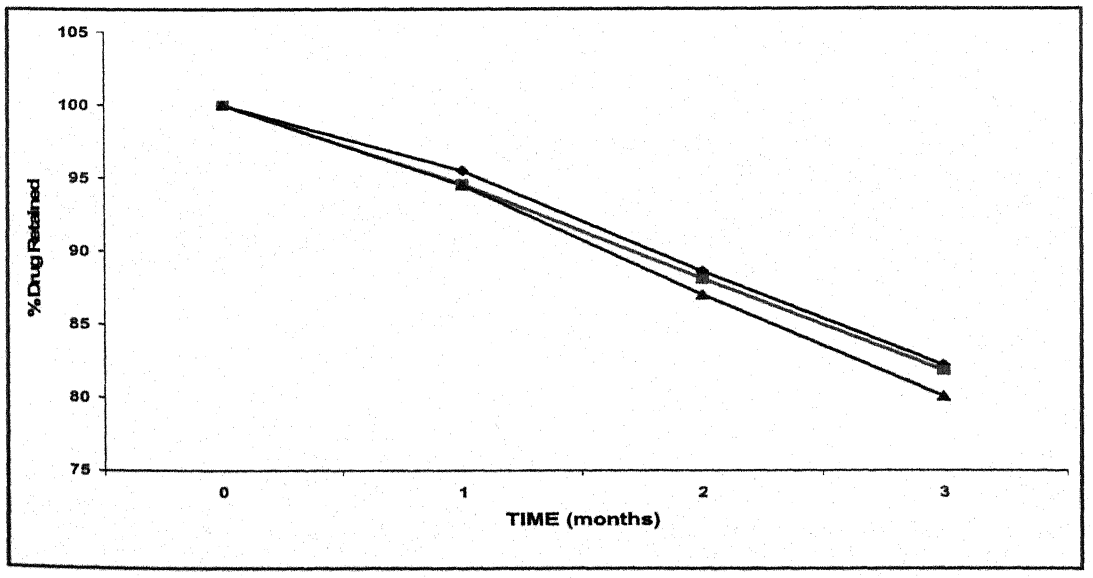


Figure-146(b): Stability of 40% Poloxamer liposomal gel at 4^0C, room temperature and 37^0C

Chapter- 12

Invivo Release Of Zidovudine In Rabbit Model

Chapter-12

INVIVO RELEASE OF ZIDOVUDINE FROM DIFFERENT LIPOSOMAL GEL IN RABBIT MODEL

12.1 TOPICAL STUDIES:

The problem with a topically applied drug is that when it is applied to the surface of the skin, It may not penetrate rapidly or extensively enough to achieve effective concentrations within the skin. In other cases the drug may penetrate the skin, but be just as quickly removed by the blood or Lymphatic systems. This may lead to systemic effects, in addition to the desired local actions. In the 1960s many non-prescription formulations designed for the topical administration of drugs were available and widely used. However, the discovery that these compounds were associated with an incidence of contact sensitization and cross-reactivity has all but eliminated their use in skin. Some topical antihistamine formulations are still available but should be used with caution because any benefit may be over shadowed by the risk of inducing an allergic contact dermatitis[181].

12.2 FACTORS CONTROLLING DERMAL DRUG DELIVERY:

12.2.1 Skin the Target Organ Anatomy and Physiology:

In pharmaceutics research on area of the specialty is devoted to develop new, controlled, site-specific drug delivery systems. With conventional vehicles there is no effective way to limit or control percutaneous absorption. Progress in pharmaceutical research has provided novel dermatological vehicles designed to control the release and to some extent the penetration and pharmacokinetic fate of the active ingredients applied topically. In contrast with currently available topical dosage forms especially if penetration promoting agents are included in the formula percutaneous absorption may lead to unintentional systemic and possibly toxic effects. The skin in an adult human body covers an area of about 2 square meters and receives about one third of the total blood supply[182]. On average every one square centimeter of skin has about 10 hair follicles, 12 nerves, 15 sebaceous glands, 100 sweat glands, 3 blood vessels and 3 million cells. The skin is referred to as a multilayered organ that can be distinguished microscopically into three major parts epidermis, dermis, and hypodermis[183].

The epidermis is a continuous stratified keratinizing epithelium which upon differentiation gives rise to the outermost flattened, dehydrated and dead keratinized layer, the stratum corneum which can be represented by a "brick-and-mortar model[184] . The bricks consist of a nucleate corneocytes filled with a matrix of keratin filaments surrounded by sulfur-rich proteins, composed mainly of involucrine Corneocytes containing gamma-hydroxylacyl sphingosines in the lipid portion of the envelope that is the intercellular spaces representing the mortar are filled by multiple lipid lamellae that unlike other biological membranes do not contain phospholipids.Instead they contain ceramides, cholesterol, fatty acids and cholesteryl esters which represent an efficient barrier to the penetration of many hydrophilic substances as well as provide a barrier against water loss from the body. The stratum corneum results from the differentiation of the epidermis cells particularly the keratinocytes microscopically different layers of epidermis each representing a unique stage of differentiation can be observed which can be classified from the dermis to the surface as follows: basal layer, stratum spinosum and stratum granulosum Beneath the epidermis resides a moderately dense fibro elastic connective tissue known as the dermis. It consists of collagen and elastin fibers forming a network within a matrix of mucopolysaccharides. The dermis plays a role in the control of passage of nutrients, it also serves as a mechanical support for the epidermis as well as a physiologic support via the blood vessels the nerve endings and the lymphatic system embedded within it[185].

12.3 MECHANISM OF DRUG PENETRATION THROUGH THE SKIN:

Penetration of the stratum corneum lipid lamellae represents the rate-limiting step governing drug transport through the skin: it has been shown that hydrophilic compounds are absorbed 1000 times more rapidly when stratum corneum is damaged. Even with intact skin a ten thousand-fold difference was noted for a range of different types of drugs depending on the structure and physiochemical properties of both the drug and the penetrating vehicle.

Two major routes for drug penetration have been postulated the intercellular route and the trancellular route. The intracellular route is the route of penetration of

small molecules via the intercorneocyte lipid rich region of the stratum corneum while penetration through the corneocytes represents the transcellular pathway. These account for about 99% of the Transepidermal routes. Shunt pathways are defined as penetration through skin appendages.

The transfollicular and transsebaceous routes. which account for less than 1 %, while the transsecrine route does not exceed 0.1%[186]. Depending on the physiochemical properties of the drug molecules. In particular the diffusion coefficient and the water- octanol partition coefficient. Compounds may penetrate the stratum corneum to the epidermis aqueous environment and then distribute to the dermis and the blood stream. Compounds that are extremely hydrophilic would not penetrate the skin while extremely lipophilic ones would be retained in the epidermis and would not penetrate deeper into the dermis. Liposomes have been demonstrated to improve penetration of certain drugs by means of drug targeting either for dermal and in some cases for transdermal delivery. However these studies have been conducted mainly in normal healthy skin. Many diseases affecting the skin may alter its permeability. For example hyper proliferation of the stratum corneum was found to increase permeability due to reduced barrier properties.

12.4 STUDY DESIGN OF IN VIVO ANIMAL EXPERIMENTS

This study was approved by the Animal ethical committee (CPCSEA)

12.4.1 Animals

Five male white rabbits 2.5 Kg, obtained from the Department of pharmacy, Bundelkhand University, Jhansi were used in this study. Rabbits were not studied until after 2-day of antibiotic therapy and a two-week environmental adjustment period. The antibiotic therapy did not affect these studies. They were kept individually in metal cages fitted with wire floors. Food and water were supplied ad libitum.

The mean zidovudine concentration versus time plots after the topical application of 10 mg Zidovudine in MLV liposomes and in the control gel containing zidovudine was plotted.

12.5 EXPERIMENTAL PROCEDURE:

12.5.1 Invivo release studies:

In this study, the gel formulation used as the control and was prepared by simple dispersion of polymers in water and 10mg of zidovudine. The animal research study, approved by the animal ethical committee (CPCSEA). Five white rabbits, mean ± weight 2.5kg± 0.05kg, were studied. Before investigations, each rabbit was housed individually in a metal cage with a wire floor. Food and water was supplied ad libitum. During initial catheterization and dosing, each rabbit was placed briefly in rabbit holder and then returned to its own holding cage. Two days before each study, a 10cm x 10cm area on the back of each rabbits was shaved using an electric shaver. One day before each study, a depilatory was applied for 10 minutes to the shaved area on the back and to ears, and then thoroughly washed off, to ensure complete removal of the hairs.

For blood sampling, a catheter was inserted in to the ear artery. After 0.5ml of blood was withdrawn and discarded, a 1.5 ml sample was collected as the predose control and placed in a centrifuger with no additives. The catheter was flushed with 2 ml of 0.9% sodium chloride followed by 0.2 ml heparin solution to avoid the coagulation.

1g of liposomal gel of Carbopol 940, HPMC K4 100, Citosan,Poloxamer containing 10mg zidovudine were applied to the defined area on the rabbit back. The rabbit was kept in the rabbit holder during the 24 hours study to prevent it from licking its back and dislodging the catheter from the ear artery. The blood sampling was repeated, as previously described for the predose sample, at 0.5, 1 2, 3, 4,6,8,16,24 hours. After centrifuging for 15 minutes at 3000rpm, plasma zidovudine concentrations were analyzed using spectrophotometrically at 267nm. *In-vivo* animal studies to be closer to the application of liposomes in humans. An appropriate viscosity of liposomal preparation is required. Carbopol940 2% and HPMC K4 100 2%, Chitosan 2%, Poloxamer40% showed slower release than Carbopol940 1% and HPMC K4 100 1%,Chitosan 1%, So Poloxamer Carbopol940 2% and HPMC K4 100 2% Chitosan 2%, Poloxamer40% was taken for the animal *in-vivo* studies.

Table-95: Invivo percentage release of HPMC K4 100 2%, Carbopol940 2%, Chitosan 2%, Polaxamer 188 2%.

S. No.	Time in hours	Cumulative % Release of HPMC K4 100 2%	Cumulative % Release of Carbopol 940 2%	Cumulative % Release of Chitosan 2%	Cumulative % Release of 40% Polaxamer 188
1	0	0	0	0	0
2	0.5	6.52	5.63	4.38	5.86
3	1	8.45	7.58	5.71	8.01
4	1.5	11.23	9.98	6.83	10.23
5	2	14.25	11.42	8.64	12.45
6	4	21.54	16.58	11.01	15.1
7	6	25.64	20.36	13.52	19.54
8	8	28.65	25	16.57	24.87
9	10	31.87	28.75	18.14	27.46
10	12	33.5	29.21	21.68	30.1
11	14	34.9	30.84	23	32.45
12	16	35.6	31.66	26	34.89

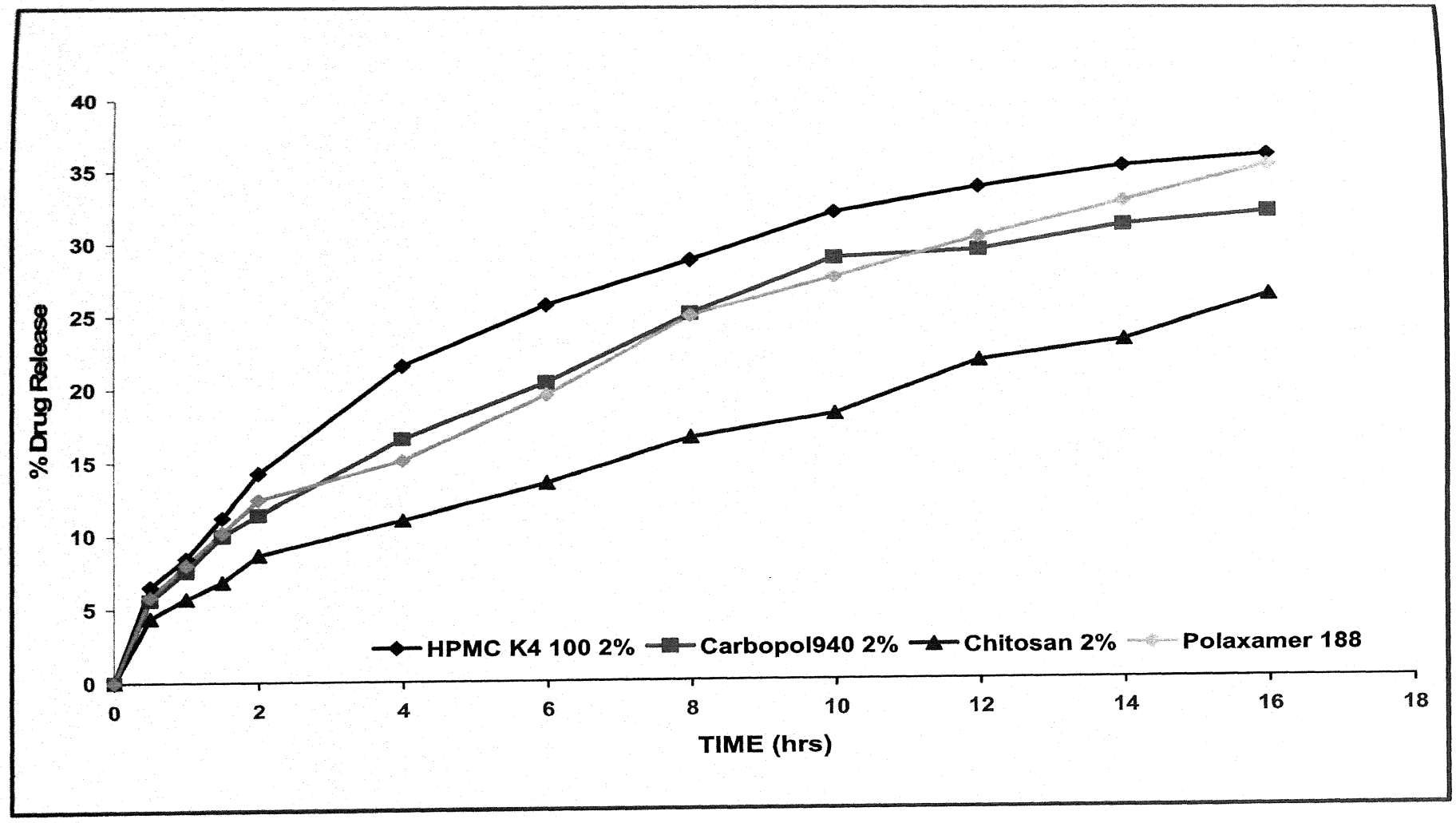


Figure-146: Invivo Cumulative percentage release of HPMC K4 100 2%, Carbopol940 2%, Chitosan 2%, Polaxamer 188 2%, Liposomal Gel containing zidovudine.

The slower release is due to the skin permeation of the drug because the drug has to cross several barriers in the skin. The prolonged release can be achieved by their incorporation in vehicle suitable for topical self-administration. The prolonged retention on the skin is often required for the desired therapeutic effect. Research efforts have been directed to using hydrophilic polymers with adhesive character to improve drug delivery via skin. It has already proven that liposomes are fairly compatible with polymers derived from cross-linked polymers. Therefore the research work confirmed to choose a gel made of Carbopol 940, HPMC K4 100, Chitosan, Poloxamer polymers as a vehicle for liposomes.

The maximum concentration of Zidovudine in MLV liposomes prepared by thin film hydration method at 1.0 hour was around 8%. Significantly lower than the plain gel-containing drug, which yielded Zidovudine serum concentration at 1 .O hour of 35%. The lower concentration of Zidovudine detected in the blood after the topical application of MLV liposomes is probably due to the intrinsic action of MLV liposomes acting as a reservoir in skin stratum and releasing the drug slowly over time into the skin. Hence gel-containing drug might release drug very soon and penetrate deeper into the skin layers and come in contact with blood vessels in the dermis resulting in a higher serum concentration. There is no effective way to limit or control percutaneous absorption in case of conventional vehicles like gel formulations. Drug penetrated quickly into the skin and was removed quickly by the blood and/or lymph systems that resulted in a higher serum concentration.

In one study demonstrated that hydrocortisone prepared in a Liposome gel and applied to the back of hairless mice with normal skin and stratum Corneum-removed skin at a dose of 1 mg, resulted in a reduced loss of hydrocortisone from the skin. Compared with a conventional ointment formulation, Drug concentrations in the viable skin were maintained at a nearly constant level for over 8 hours by applying a liposome gel formulation. As a result 5 fold higher viable skin drug concentrations were obtained from liposomes than from a conventional ointment. The plasma level of hydrocortisone at 4 hours from liposome gel was only 25% of that obtained from the ointment, when the drug was applied to the stratum corneum - removed skin[187,188].

Chapter- 13

Result and Discussion

Chapter-13

RESULT AND DISCUSSION

IR Studies:

Figure 67-78 and Tables 3-9, shows that there is no interaction between the drug (Zidovudine), polymers (HPMC, Chitosan, Carbopol and Poloxamer) and lipids (PC and CH) used in the formulations.

Preparation of standard curve:

Spectrophotometric method of analysis was chosen for identify whether the drug obeys Beers laws. Dilutions of the drug (1-6mcg/ml) and the absorbance were measured by UV spectrophotometry at λ_{max} of 267nm. The absorbance Vs concentration was plotted which yielded a straight line, showing that the dilutions of the drug obeyed Beers law.

Formulation of liposomes:

The formulation method employed for the preparation of liposomes was thin film hydration method. Various ratios of PC and cholesterol were taken i.e. 1:1, 2:1, 5:2 and 10:3 for the preparation of liposomes.

On observation of the formulated liposome for its entrapment efficiency it was seen that thick film of lipids which gave liposome with less entrapment of zidovudine. The thin film showed better entrapment than the thick film (i.e.) 59±1.6% compared to the entrapment efficiency of 35±0.5% in case of thin film. The encapsulation of zidovudine in the liposomes is selected to the overall volume of aqueous phase.

When considering the pharmaceutical application and delivery of all the controlled formulations, almost all the reported encapsulation efficiencies are less than 100%.[189]

Analysis by DSC :

DSC analysis reveals a change in the transition temperature, which given ideas on the liposomal stability, permeability and drug entrapment.

Drug release from conventional liposomes:

On studying the drug release from conventional liposomes it was seen that 98.6% of the drug was released within 8 hours.

Determination of encapsulation efficiency:

To determine the encapsulation efficiency of the liposomes, the vesicles were ruptured using Triton X-100. At the 5% concentration Triton X-100 solubilizes 100% lipid bilayer.

Validation of method of spectrophotometry :

The recovery studies showed that the procedure for determination of zidovudine is valid.

Osmotic study:

The osmotic studies revealed that under hypotonic conditions the plot deviated from the linear relationship since the liposomes were lysed, the solute leaked and the liposomal membrane lost its membrane barriers function.

Stability of Liposomes in Plasma:

The stability of empty liposomes in plasma was studied and graphs plotted between absorbance vs time in hours showed that when the liposomes were stored from a long period of time and samples analyzed spectrophotometrically showed that after the 12th hour aggregation of the vesicles had taken place thereby increasing the absorbance indicating instability which is shown in figure-49.

DRUG RELEASE FROM LIPOSOMES :

Effect of temperature:

Drug release from conventional liposomes at different temperature shows that there is no significant effects on zidovudine release at 37-39°C but with higher temperature in the range of 50°C the release was found to be increased which may be done to the increase in temperature leading to the increased value of diffusion of the drug. The results are shown in table-21. In-vitro testing provides an invaluable tool for investigating drug release mechanisms[190].Release of drug from liposomes

usually results in a burst effect phenomenon due to accumulation of drug molecules at the liposomes surface during storage[191].

Effect of stirring speed:

The stirring speed is varied based on the characteristics of drugs and testing devices[192]. The effect of stirring speed on the release of drug from liposomes was studied which shows that an optimum speed of 100 rpm was suitable for the gradual release of liposomes. Increase in speed to more than 200 rpm destroyed the dialysis bag. The results are shown in table- 27 & figure-55.

Effect of size on drug release:

Release kinetics of drug from liposome showed that particle size affects the release kinetics. The delivery of aqueous drugs to cells was improved by formulating unilamellar liposomes.1100 nm in diameter owing to their great internal diameter[193]. The liposomes which are very small in size showed better release when compared to liposomes of larger size. This may be due to the increase in surface area when the particles are smaller in size. The results are sown in table-33 & figure-60.

Effect of pH on drug release:

The drug release mechanism from liposomes can be influenced by the various physical or chemical factors. (pH, addition of salts).[194] On studying the drug release at different pH (pH 3, 5.0,7 and 8) it was seen faster release was observed at pH 3 this may be due to fusion of liposomes at this pH. The results are shown in table-39 & figure-65.

Effects of salts on stability [194]:

On addition of salts to the colloidal liposomal preparation, aggregation occurs forming singlet, doublets, triples, quadruplets and pH high salt concentration total mass aggregates occurred. The results are shown in figure-70-76.

Effect of temperature on liposome size enlargement:

The aggregation of liposomes is due to Brownian motion. While increasing the temperature there will be increasing in motion of liposome vesicles.

Reversible aggregation by increasing temperature has been reported in lipid systems.[195]

The reaction limited clusters aggregation process the average cluster size grows exponentially with time. After aggregation liposomes form large vesicles. So this study shows at 4°C, liposomes were more stable. The results were shown in the table-45 & figure-77.

Effect of pH on liposome size enlargement:

The influence of pH on liposome size enlargement was studied, it was seen that at pH above 5-8, there is no significant change in size enlargement but the decreased pH (pH-3) the rate of membrane fusion may occur in neutral liposomes. The results are shown in table-46 & figure-78.

Effect of $CaCl_2$ on liposomes size enlargement:

In the range of 0.001M to 0.01M concentration of $CaCl_2$ there is no significant change in size enlargement. Above 0.01M i.e. 0.1M $CaCl_2$ may induce aggregation. The results are shown in table- 47& figure-79.

Stability studies of conventional liposome suspension:

Stability study results showed that the formulation can be stored at 4°C.

Effect of cholesterol on zeta potential:

Phosphotidylcholine liposome membrane possessed a negative charge at pH7 suggesting that the natural phosphotidylcholine included a small amount of acidic components[196].

The results of this study showed that zeta potential of naked liposomes was -22.2mV. Zeta potential started to decrease with decreasing concentration of cholesterol into the phospholipids and eventually becomes a constant at -5.91mV.

Carbopol gels: Effect on drug release

The liposome containing the drug was incorporated into the gels and the release characteristics were studied.

The rate of release from C940 was slowest when compared with C971 and C974. The release was fastest from the least cross linked polymer and slowest from the polymer C940.

On varying the concentration of Carbopol 971P it was found that within 24 hours only 66.5% of drug was released from 3% whereas 98.89% of drug was release from 1% C971and 85.72% from 2%C971P.

On comparison of the release profile of Carbopol 974P (1%, 2%,3%) 84.01%, 70.23% and 61.2% were released from 1%, 2%, 3% carbopol 974P at the 24 hour.

On comparison of the release profile of Carbopol 940 (1%, 2%, 3%) 43.59%, 40.87, 32.7 were released at the end of 25th have by 1%, 2% and 3% Carbopol 940P.

Comparison of the stability of liposomes and Liposomal gel :

On comparison of the stability of liposome and liposomal gel 2%, 3% found that the stability of liposomal gels at 4% room temperature and 37^{0}C was more than conventional liposomes shared at 4^{0}C, room temperature and 37^{0}C. This was show in table-67& 69.

Release of Zidovudine from HPMC Gel:

Liposomal containing Zidovudine was incorporated in to HPMC gel (1%, 2%, 3%). The release from HPMC 2% and 3% was 98.47% and 98.78% whereas the percentage release from conventional liposomes was 98.64%.

Stability of 2% HPMC K4 100 liposomal gel at 4^{0}C, room temperature and 37^{0}C:

Stability studies results shows that the liposomal gel stored at 4^{0}C showed maximum stability and so 4^{0}C can be the storage condition for the formulation.

Stability of 3% HPMC K4 100liposomal gel at 4^{0}C, room temperature and 37^{0}C:

Stability profile of 3% HPMC K4 100 liposomal gel stored at various conditions 4^{0}C, room temperature and 37^{0}C revealed that 4^{0}C is the most favorable storage condition.

Stability of 2% HPMC K 15 in liposomal gel at 4°C, room temperature and 37°C:

Stability profile of 2% HPMC K 15 showed that of room temperature of % drug retained was 88.2% whereas at 4°C it was 93.0% which showed that 4°C was the most favorable condition for storage.

Stability of 3% HPMC K 15 liposomal gel at 4°C room temperature and 37°C:

The studies revealed that at 4°C, 93.5% of drug was retained whereas at room temperature 89% of drug was found remaining which suggested that 4°C was ideal for the storage.

Incorporation of liposome into chitosan gel:

Chitosan gel 1%, 2%, 3% was prepared and percentage release was studied. It was found that at the 24th hour have 73.5%, 61.1%, 46.7%, was released by 1%, 2% and 3% chitosan gel.

Stability of 1% chitosan liposomal gel at 4°C, room temperature and 37°C:

Stability studies showed that the formulation can be stored at 4°C and 37°C, as the % of drug remaining was 90.2% at the end of three months.

Stability of 2% chitosan liposomal gel at 4°C, room temperature and 37°C.

Stability studies showed that the formulation can be stored t 4°C and 37°C as the drug remaining at this temperature was 94.1% and 90.21%.

Stability of 3% chitosan liposomal gel at 4°C, room temperature and 37°C:

The studies showed that the formulation can be showed at 4°C and 37°C as 94.5% of the drug was retained at 4°C and 90.8% was retained at 37°C.

Poloxamer gels incorporation of Zidovudine liposome into poloxamer gels into % drug release:

Zidovudine liposomes were incorporated into poloxamer gels (30% & 40%) and the % release was calculated. It was found that at the 24th hour 97% of drug was released from 30% poloxamer gels and 89.6% of drug was released from 40% poloxamer.

Stability of 30% poloxamer:

Stability analysis of 30% poloxamer liposomal gel showed that the suitable storage environment of the formulation was at 4°C.

Stability analysis of 40% poloxamer gel:

Stability analysis showed that room temperature was suitable for the storage of the formulation.

In-vivo release of zidovudine from liposomal gel in Rabbit model:

In-vivo release studies were preformed in rabbit model. 1g of liposomal gel, of carbopol 940, HPMC K4 100, Chitosan, Poloxamer containing 10mg of Zidovudine were applied to the rabbit skin and blood sampling was carried out at 0.5, 1,2,3,4,6, 8,16,24 hours. The results showed that maximum concentration of Zidovudine liposomal gel (HPMC, Carbopol, Chitosan, Poloxamer) was around 5-8% whereas gel containing drug yielded zidovudine serum concentration of % around 25%. Hence this study showed that 5 fold higher viable skin drug concentrations were obtained from liposomes then from conventional formulation.

Chapter- 14

Summary and Conclusion

Chapter-14

SUMMARY AND CONCLUSION

Investigation was made upon the preparation. Characterization, stability and drug release characteristics of conventional liposomes and liposomal gel formulated. Liposomes in the size range of 601 nm to 1100 were obtained. The measured zeta potential of liposomes as a function of the concentration of cholesterol and phospholipids showed that the zeta potential start to decrease with decreasing concentration of cholesterol.

Various parameters affecting the formulations were studied which includes temperature, stirring speed, effect of salt. All these parameter had little impact on the formulation. The release studies and the stability analysis were performed.

Further on the basis of stability and release characteristics it was seen that Chitosan (2%) liposomal gel, HPMC (2%) liposomal gel, Carbopol (2%) liposomal gel and poloxamer (40%) liposome gel showed better result.

In-vivo studies were carried out using the liposomal gel. It was compared with the gel containing Zidovudine. The liposomal gel of Zidovudine showed promising results when compared with the normal gel.

This indicates that the findings may be utilized for the improvement of the therapeutic efficacy of liposomal gel as drug delivery systems for Zidovudine.

Chapter- 15

Future Plan

Chapter-15

FUTURE PLAN

1. The design and development of liposomes based delayed release formulation for oral administration is to be tried and detailed studies have yet to be done on it.
2. The mechanisms of drug absorption and improvement of the absorption efficiency and the timing requirements is yet to be focused.
3. Fate and biodistribution of liposomes is to be studied.
4. The influence of surface modification to understand the liposome interaction and drug release mechanism is yet to be analysed.
5. Adsorption mechanism at the liposome surfaces and their influence in stability and drug release warrants further investigations.
6. The mechanisms of drug transport through the cell membrane after the liposome has interacted with the cell surfaces and membrane permeable mechanisms.

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List of Publications

PAPERS PUBLISHED :

1. Cyanocobalamine resinate and its stability studies Published in Asian Journal of Chemistry.
2. Formulation and evaluation of Buccal Patches of Ketrolac tromethamine published in Biosciences, Biotechnology, research Asia
3. Formulation and evaluation of Omeperazole Niosomes published in Biosciences, Biotechnology, research Asia.
4. Formulation and evaluation of Nanoparticles of Mitomycin-C published in Journal pharmaceutical research.
5. Nanoparticles as drug delivery system published in The Pharmacist bi annual issue
6. Applications of liposomes: A Review published in The Pharmacist bi annual issue

PAPERS ACCEPTED :

1. In-vitro Release Behavior and Stability of Liposomal Formulation of Zidovudine accepted in Advances in Pharmacology and Toxicology
2. In-vitro and In-vivo Release of Zidovudine from Different Liposomal Gel in Rabbit Model accepted in International Journal of Pharmacology and Biological Sciences.
3. Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope accepted in Indian Journal of Applied Life Sciences.
4. Natural Soya Lecithin as a Drug Delivery-A Review accepted in Plant Archives.

PAPERS COMMUNICATED :

5. Evaluation And Development of Mucoadhesive Buccal tablet of Salbutamol Sulphate communicated in Journal of pharmacy and Science.
6. Preparation and Evaluation of Aceclofenac Micro spheres communicated in Asian Journal of Pharmaceutics.
7. Development and invitro evaluation of Liposomal topical delivery system for Cyclopirox Olamine communicated in International Journal of Pharmacology and Biological Sciences.

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To,
Prof./Dr. S. Palani
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Dear Dr. Palani

I am pleased to inform you that your paper, "In Vitro release Zidovudine" by Sharma & Palani
has been accepted for publication in the Journal "Advances In Pharmacology And Toxicology."

It will be published in Vol. 8 No. 3 of 2007

With best wishes.

Yours sincerely,

(Ram Prakash)

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Invitro Release Behavior And Stability Of Liposomal Formulation Of Zidovudine

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ABSTRACT:

The objective of the present study was to develop the release kinetics of liposome encapsulated material in the presence of phospholipids. To determine the factors influencing encapsulation of zidovudine in liposomes and to optimize the encapsulation parameter. Zidovudine was encapsulated in multilammellar liposomes, prepared using Phosphotidyl choline and cholesterol. The effects of method of preparation, type of vesicle formed, charge of the vesicle by using zeta sizer, Concentration of cholesterol in encapsulation of zidovudine in liposomes were investigated. Also releases of zidovudine under various conditions like temperature, Stirring speed and invitro drug release from liposomes was studied using a dialysis method by using the dialysis bag.

Keywords: Liposomes, Zidovudine, Liposomal Gel, Topical application

INTRODUCTION:

Liposomes, after three decades of research are still gaining knowledge with special emphasis more recently on their use as drug carrier systems (**D.D.Lasic, 1993**). Liposomes have been successfully used as delivery vehicles to improve therapeutic efficacy and reduce toxic effects (**G.Gregoriadis, 1988**). For therapeutic purposes they must be loaded with drug substance. This is more essential achieved with amphiphile molecules like phospholipids and cholesterol as they have a tendency to be incorporated in the liposomal membrane. In contrast, hydrophilic molecules must be encapsulated in the aqueous interior, which in general, cannot easily be performed in an efficient manner (**M.J.Ostro1987**). A variety of liposomes preparations were introduced and they have to fulfill the basic requirements (a) Produced liposomes should be homogeneous (b) Efficient of encapsulation of hydrophilic drugs. Where as thin film hydration technique achieve high efficiency and easy method to produce in the laboratories (**R.L. Juliano et al**). The concept of this project was fairly to entrap hydrophilic drugs like zidovudine. The ratio of volume inside the liposomes compared to the total aqueous volume of the preparation is encapsulation efficiency. The increasing lipid concentration more liposomes per unit volume of the preparation are formed. When phospholipid dispersed in aqueous medium at certain concentrations results in highly viscous dispersions up to semisolid consistency. So, the phospholipid, cholesterol and hydration times were changed to achieve maximum entrapment efficiency.

Zidovudine, the first anti HIV compound approved for clinical use is widely used for the treatment of AIDS either alone or combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of zidovudine its dose dependant hematological

toxicity, low therapeutic index, short biological half life and poor bioavailability (**K.D.Kieburtz et al**). Zidovudine available in the market as conventional tablet form. After oral administration, it is rapidly absorbed from GIT exhibiting a peak plasma concentration of 1.2µg/ml at0.8 hours(**R.W.Klecker et al**). In the systemic circulation, it is first converted to Azidothimidine triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of zidovudine is 4 hours. To maintain the constant therapeutic drug levels and to target the virus an adequate zero order delivery of zidovudine required. Therefore a simple method was choosed to study the release kinetics of liposomal encapsulated material such as zidovudine.

PREPARATION OF LIPOSOMES:

Multilammellar liposomes were prepared using the thin film hydration method (**Bangham et al**). Accurately weighed quantity of drug, Phosphotidyl choline, Cholesterol. Phosphotidyl choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flasks. The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing saline for 2 hours until vesiculation was completed.

Estimation of Entrapped Drug in Liposomes

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15 000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained. The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation(**Kulkarni et al**). The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant. There are two methods to find out the entrapment efficiency, the first one is by using the methanol and second one is by using detergent like TritonX-100.

The amount of drug exactly present within the liposomes was also analyzed by dissolving the liposomes in methanol to countercheck the percentage drug entrapment and to arrive at a mass balance. The analysis of drug in liposomes was carried out using the empty liposomes dissolved in methanol as blank in order to nullify the interference of the excipients.

Encapsulation efficiency:

The pellets obtained from centrifugation were resuspended in 0.9% w/v saline solution. The influence of detergent on liposomes solubilisation was determined by dissolving

300ul of liposome suspension with 0.5%, 1%, 2%, 3%, 4%, 5%, and 6% with different concentrations to find out the exact concentration of Triton X-100 detergent. The detergent solution was then gently heated for 5 minutes. The clear solution was quantitatively analyzed for zidovudine by spectrophotometery at 267nm.

Dialysis bag method:

Zidovudine release from liposomes was determined by using the dialysis bag. The dialysis bag membrane is permeable to release medium and it can allow the drug such as zidovudine. Before using the dialysis bag was soaked in water for around four hours to remove the contaminants and debris in the membrane. A 4.0 ml of zidovudine liposomal suspension and empty liposomal suspension separately was taken in to beakers containing 100ml saline solution was the drug release medium. The medium temperature was maintained at 37°C prior to use, in order to dearate the medium, the stirring speed and temperatures were maintained at 100± 10 rpm and 37 ± 0.5°C respectively. An aliquot of 2ml sample was withdrawn at different time intervals for the determination of zidovudine concentration by U-V spectroscopy at 267 nm. At the same time, same volume of water was replaced to maintain the sink condition.

The empty liposomes was taken to found that the absence of zidovudine, no λ max at 267 nm was measured in solvent, which indicates that the liposomes constituents do not interfere with the measurement of zidovudine concentration. The above release test was to conform the interference of phospholipids or other substances.

Optical microscopy:

Zidovudine liposomal dispersion was examined by optical microscope (Magnus-DLX-DX, Olympus Labs) before and after the release of drugs from liposomes.

Transmission electron microscopy:

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

Determination of zeta potential:

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer.The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential(**D.J.Crommelin et al**).

Stability of the liposomal dispersions:

The drug loaded liposomal dispersions were stored at 4°C, 25°C and 37°C. The liposomes were observed under microscope for the size and appearance at the end of every week. The stability test was conducted for 4 weeks to find out the drug stability in the vesicles.

RESULTS AND DISCUSSION:

The ratio of the Phosphotidyl choline, cholesterol, hydration times and rotation time of the flask were varied to get the maximum entrapment of zidovudine a hydrophilic drug.

Table-1 : Composition, Hydration time, Mean particle size

S. No.	PC: CH (mg)	Drug (mg)	Hydration time	Rotation time of the flask	EE
1.	200:200	10	02	50	35±0.8
2.	200:100	10	04	100	42±1.2
3.	200:80	10	06	150	53±1.0
4.	200:80	10	08	150	59±1.6
5.	200:60	10	08	150	51±1.8

PC- Phosphotidyl Choline

CH-Cholesterol

EE-Entrapment Efficiency

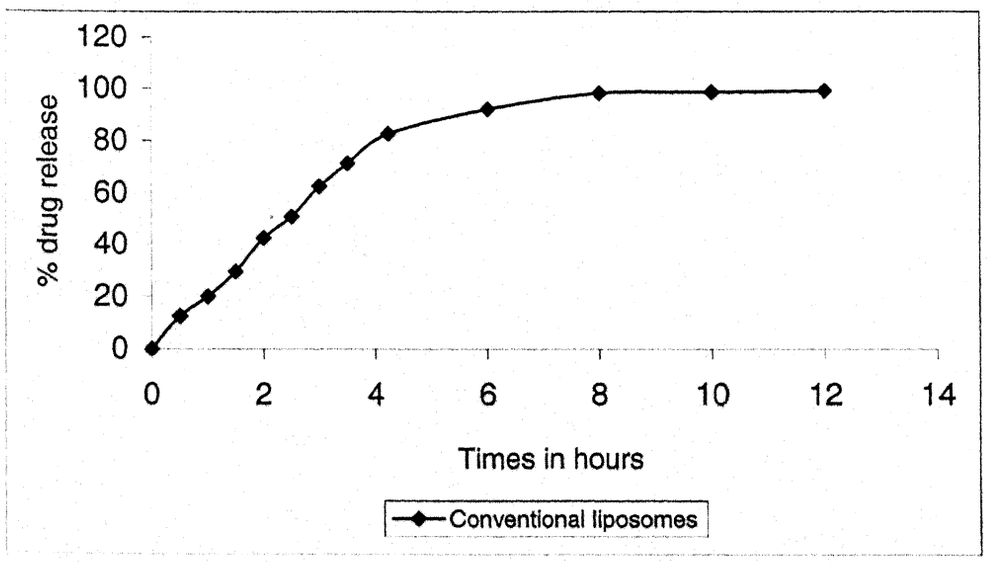
(n=3)

The data of the table-1 showed that the thick film of lipids which gave liposomes with less entrapment of zidovudine. The thin film showed better entrapment than thick film i.e. 35±0.8% compared to the entrapment 59±1.6% obtained in liposomes prepared by thin film. The effects of the formulation variables, the lipid phase composition PC/CH and hydration condition on drug entrapment efficiency and mean particle size of liposome vesicles. Decreasing amount of cholesterol in the lipid phase and increasing the drug concentration, the entrapment efficiency of zidovudine in to liposomes increased(**G.Gregoriadis et al**). The drug substance has a slight affinity for the lipid phase. The encapsulation of the zidovudine in the liposomes is related to the overall volume of aqueous phase encapsulated during liposomes formulations. Triton-X 100 is used to separate drug from the liposomes, 5% is suitable. Below and obove5% showed less drug concentration. So, a 5% triton-X 100 concentrations is suitable to find out the drug entrapment in the liposomes. The prepared liposomes are of similar size, with the mean diameter of 1100nm and the charge of the liposomes was observed that the particles are negatively charged, the range is –22.2. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). The DSC studies were conducted to obtain evidences of the drug entrapment by the liposomes. The transition temperature was increased, so it confirms the drug entrapment. The phase transision temperature can give good clues about liposomal stability, permeability and whether a drug is entrapped in the bilayered or in the aqueous compartment (**M.Weiner et al**). The drug release of the conventional liposome formulations is present in the table-2 and fig-1. It was found that 98.64% of encapsulated drug was released during a period of 8 hours, in 37°C temperature. The hydration condition, an increase of hydration time, led to a slight increase of drug entrapment showed in table-1.

Table-2 : Drug release from conventional liposomes

S. No	Time in hours	% Drug release from conventional liposomes
1.	0	0
2.	0.5	12.5
3.	1	21.45
4.	1.5	29.56
5.	2	38.2
6.	2.5	50.74
7.	3	62.48
8.	3.5	71.25
9.	4	84.6
10.	6	92.01
11.	8	98.23
12.	10	98.56
13.	12	98.99
14.	14	98.74
15.	16	98.01
16.	18	99.01
17.	20	98.38
18.	22	98.12
19.	24	98.64

Fig-1 Percentage drug release from conventional liposomes



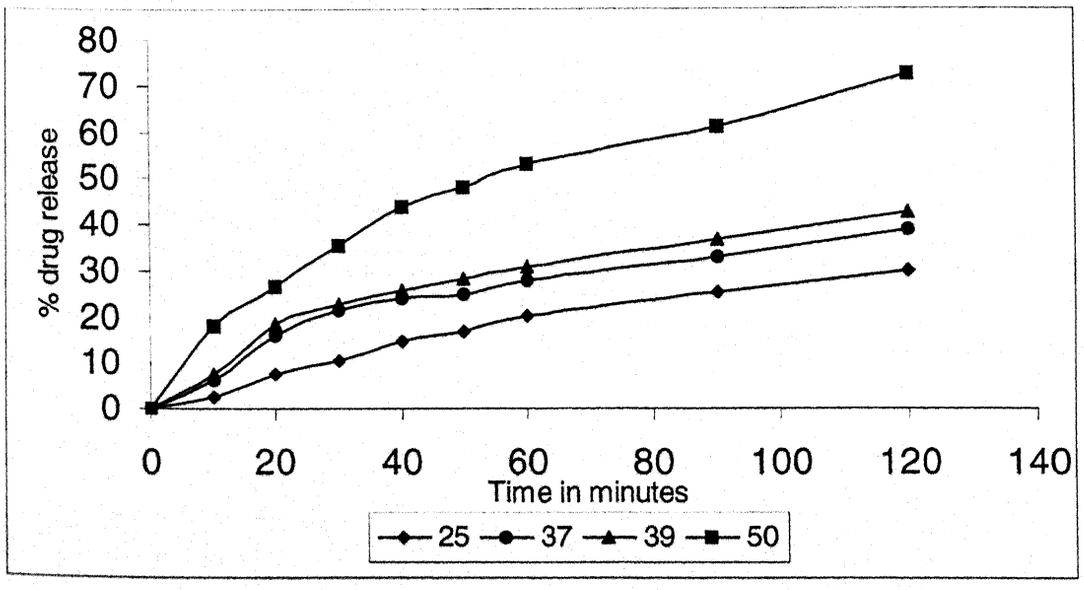
Effect of temperature on the release of drugs from liposomes :

The temperature of the release medium may alter the viscosity of the liposomal suspension. Generally the human body temperature is 37°C. How ever in the clinical cases, such as fever, the variation of temperature can change the drug release when liposomes are sensitive to the temperature (**D. Papahadjopoulos et al**). The fig-2 shows that increasing temperature of the medium increases zidovudine release from the conventional liposomes. The releases studies were conducted for 2 hours to find the behavior of liposomes at different temperature. Stirring speed kept constant at 100rpm. The temperature of the medium has no significant effects on zidovudine release from liposomes in the range37-39°C. Which means that drug release from the liposomes in non-temperature sensitive invitro at body temperature. But with increased temperature, the zidovudine release rate increased. The effect because of the rising temperature increases the value of diffusion.

Table-3 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

S.No.	Time in minutes	% Drug released at 25°C	% Drug released at 37°C	% Drug released at 39°C	% Drug released at 50°C
1.	0	0	0	0	0
2.	10	2.53	6.24	7.51	18.02
3.	20	7.42	16.03	18.62	26.32
4.	30	10.54	21.54	22.67	35.45
5.	40	14.65	23.99	25.87	43.87
6.	50	16.87	24.87	28.36	47.85
7.	60	20.41	27.8	30.84	53.01
8.	90	25.28	32.69	36.44	61.22
9.	120	29.98	38.54	42.57	72.42

Figure-2 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C



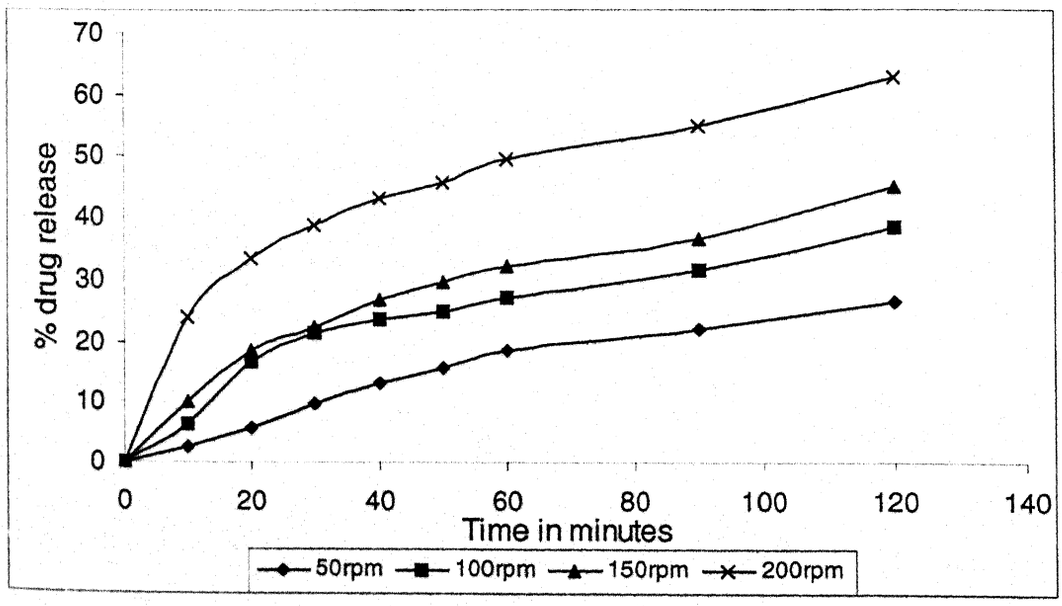
Effect of stirring speed on the release of drugs from liposomes:

The drug release test is conducted using a beaker with a magnetic stirrer. How ever for the liposomes, there is no detailed test in the official books. The stirring speed is varied based on the characteristic of drugs and the testing devices. The effect of stirring speed on the zidovudine release from liposomes is shown in fig-3 and table-4. It was shown in figure and table that increased stirring speed increase the rate of drug release. In addition with an increased stirring speed, the equilibrium time for drug release from conventional liposome decreased gradually. If a constant speed applied to the release device, the diffusion layer becomes thinner and drug release rate become larger. An increase in speed leads to reduction in the thickness of the diffusion layer. When the stirring speed 200 or more than two hundred and the dialysis bag may also be destroyed. Therefore an optimal stirring speed was 100rpm.

Table-3 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C

S.No.	Time in minutes	% Drug released at 25°C	% Drug released at 37°C	% Drug released at 39°C	% Drug released at 50°C
1.	0	0	0	0	0
2.	1	2.53	6.26	10.21	24
3.	2	5.64	16.73	18.45	33.3
4.	3	9.68	21.52	22.48	38.7
5.	4	13.25	23.79	26.79	43.1
6.	5	15.64	24.89	29.62	45.8
7.	6	18.72	27.26	32.14	49.4
8.	7	22.02	31.62	36.54	54.8
9.	8	26.58	38.54	45.23	62.9

Figure-3 : Zidovudine release from conventional liposomes at 25°C, 37°C, 39°C, 50°C



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Invitro And Invivo Release Of Zidovudine From Different Liposomal Gel In Rabbit Model

P.K.SHARMA, S.PALANI*, R. IRCHHIAYA
Institute of Pharmacy, Bundelkhand University, Jhansi-284003

ABSTRACT:

The objective of the present study was to develop a liposomal gel for the topical application of Zidovudine an anti HIV drug and capable to efficiently deliver entrapped drug during an extended period of time. Zidovudine was entrapped in the liposomes composed of Phosphotidyl Choline and cholesterol in the ratio of 200mg: 80mg and prepared by thin film hydration method. Liposomes preparation was characterized and compared for particle size mean dispersion, entrapment efficiency, DSC, Zeta potential and tested for in-vivo release in rabbits. In-vitro release studies also performed to compare the in-vitro and in-vivo release of Zidovudine. To achieve Application viscosity of liposomes and to further improve their stability than conventional liposomes. The liposomes prepared by thin film hydration method was incorporated in the bioadhesive gels like 2%, 3% of Carbopol 940, 2%3% HPMC K4 100. All the performed experiments confirm the applicability of liposomes as novel drug delivery carrier system for the topical application of Zidovudine.

Keywords: Liposomes, Zidovudine, Gel, Topical application.

INTRODUCTION:

Topical delivery systems for systemic administration of several drugs have been extensively investigated over last decade (**M.Schafer-Korting et al**). The topical dosage form attempts are being made to utilize drug carriers that ensure adequate of the drug with in the skin in order to enhance local and minimize side effects (**F.P.Bonina et al**). Advantage of the topical route of drug administration (a) avoidance of first pass metabolism (b) reduction in the incidence (c) Severity of GIT effects (d) Easiness of application (e) Complete privacy of the therapy for the patients. Liposomes are becoming increasingly important as carriers of biologically important molecules in living system. (**Tyrell *et al.*, 1976; Papahadjopoulos, 1978; Gregoriadis,1980; Roerdink *et al.*, 1981**). The application of liposomes as drug carrier on the skin surface has been proven to efficient in the delivery of liposomes entrapped drugs to and in to the skin. Liposomes applied on the skin, may act as a solubilizing matrix for poorly soluble drugs and as well as local depot for sustained drugs release (**M.J.Ostro1987**). Summarily, topical liposome formulation could be more effective than conventional formulations. Topical application of gel form, hydrophilic polymers are considered to be suitable thickening agents. The polymer, which forms the gel matrix, could influence the stability as well as the release rate of incorporated drugs.

Carbopol polymers, a well-known polymer acrylic acid cross-linked with polyalkenyl ethers. Each particles of Carbopol can be viewed as a network structure of polymer chains

interconnected via cross-linking. Carbopol readily absorb water, get hydrated and swell (**R.H.Briede,1983**). In addition to its hydrophilic nature, its cross-linked structure and it essentially insoluble in water makes Carbopol a potential candidate for use in controlled release drug delivery system (**A.T.Florence et al**).

Hydrophilic polymer gel matrix systems are widely used in controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost effectiveness, and broad U.S. Food and Drug Administration acceptance Hydroxypropyl methyl cellulose (HPMC), which is commonly used in hydrophilic matrix drug delivery systems, is mixed alkyl hydroxyl alkyl cellulose ether containing methoxyl and hydroxypropyl groups. The hydration rate of HPMC depends on the nature of these substituents, such as the molecular structure and the degree of substitution. Specifically, the hydration rate of HPMC increases with an increase in the hydroxypropyl content. The solubility of HPMC is pH independent.

Previous studies investigated that application of liposomes containing drug is possible to extend the release rate of water-soluble drugs. Continuing that research, here we report the development of a bioadhesive liposomal gel containing Zidovudine for the topical applications.

MATERIALS AND METHODS:

Materials:

The materials used in the study were Zidovudine a gifted sample of Alkem Laboratories Ltd, Raigad. Phosphotidyl choline, Qualigens, Mumbai. Cholesterol, Qualigens, Mumbai. Chloroform, Merck, Mumbai. Methanols, Qualigens, Mumbai.were Purchased. All in the study, other ingredients used were of analytical grade.

Methods:

Preparation of liposomes:

Multilameller liposomes were prepared by using thin film hydration method (**Bangham et al**)) Accurately weighed quantity of Drug, Phosphotidyl Choline, Cholesterol was taken. Phosphotidyl Choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flask. The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing 0.9%w/v Saline solution for 8 hours until vesiculation was completed.

Estimation of Entrapped Drug in Liposomes

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to configuration in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15

000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained (**Kulkarni et al**). The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant.

Transmission electron microscopy:

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

Determination of zeta potential:

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential (**D.J.Crommelin et al**).

In-vitro release studies:

Studies of drug release from liposomal gel formulations were performed using the modified Franz diffusion cell (Vertical type). Drug release from liposomes was studied by using a dialysis method (**R.Peschka et al**). Before using the membrane the dialysis membrane was soaked in distilled water at room temperature for 2 hours to remove the preservatives, followed by rinsing thoroughly in distilled water. The dialysis membrane was cut in to 2.5cm x 2.5cm^2. The dialysis membrane was cut mounted between donor and receptor compartments. The diffusion area was 1.5cm± 0.01cm2. The receptor compartment (in contact with receptor membrane) was filled with 70 ml of saline solution and the receptor compartment temperature was maintained 37°C± 0.5°C. Then donor compartment was then covered with aluminium foil to prevent evaporation of sample. At predetermined time intervals, 2.0ml of samples were withdrawn from the receptor comportment and for subsequent analysis of Zidovudine by spectrometric method at 267 nm.

In-vivo release studies:

In this study, the gel formulation used as the control and was prepared by simple dispersion of polymers in water and 10mg of Zidovudine. The animal research study, approved by the animal ethical committee (CPCSEA). Five white rabbits, mean ± weight 2.5kg± 0.05kg,were studied. Before investigations, each rabbit was housed individually in a metal cage with a wire floor. Food and water was supplied ad libitum. During initial catheterization and dosing, each rabbit was placed briefly in rabbit holder and then returned to its own holding cage. Two days before each study, a 10cm x 10cm area on the back of each rabbits was shaved using an electric shaver. One day before each study, a depilatory

was applied for 10 minutes to the shaved area on the back and to ears, and then thoroughly washed off, to ensure complete removal of the hairs.

For blood sampling, a catheter was inserted in to the ear artery. After 0.5ml of blood was withdrawn and discarded, a 1.5 ml sample was collected as the predose control and placed in a centrifuger with no additives. The catheter was flushed with 2 ml of 0.9% sodium chloride followed by 0.2 ml heparin solution to avoid the coagulation.

1g of liposomal gel of Carbopol 940 and HPMC K4 100 containing 10mg Zidovudine were applied to the defined area on the rabbit back. The rabbit was kept in the rabbit holder during the 24 hours study to prevent it from licking its back and dislodging the catheter from the ear artery. The blood sampling was repeated, as previously described for the predose sample, at 0.5, 1 2,3,4,6,8,16,24 hours. After centrifuging for 15 minutes at 3000rpm,plasma Zidovudine concentrations were analyzed using spectrophotometry at 267nm.

RESULTS AND DISCUSSION:

Liposomes have been used as a carrier system to deliver medications in to the skin in order to achieve the therapeutic effect with lower systemic absorption. Several novel carrier systems were suggested to be appropriate for topical drug delivery of liposomes. To achieve the desirable therapeutic affect of liposomes as drug carriers, they must be loaded with sufficient amount of active compounds. Therefore liposomes with Zidovudine were prepared by thin film hydration method with the effects of the formulation variables, lipid phase composition (Phosphotidyl Choline and cholesterol) and hydration time on the drug entrapment efficiency, vesicle size and mean distribution of liposomes. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). Regardless of the preparation procedure used, liposomes were of a mean diameter around 1103.509nm in diameter with the low cholesterol level. The mean particle size and charge of the liposome were increased with the increased concentration of cholesterol. How ever, liposome prepared by the thin film hydration method was of a more homogenous size distribution. This was probably a consequence of the preparation procedure, in the better case.

In this study, liposomes with Zidovudine were mixed in to 2%, 3% Carbopol 940 and 2%, 3% of HPMC and tested for in-vitro, in-vivo release of the entrapped drug by using Franz diffusion cell for in-vitro release of drug and rabbits were used to study the in-vivo release.

In in-vitro release studies, the Zidovudine conventional liposomes was released 94% with a period of 8 hours, while gel formulation released 94% of entrapped drug during a period of 22 hours. As expected, entrapped Zidovudine in a structured vehicle of Carbopol and HPMC resulted in a prolonged release rate compared to the conventional liposomes due to the restriction imposed by polymeric network of the gels. The porosity of the gel matrix allowed intact liposomes and release free drug to diffuse through the matrix in to the receptor solution (Saline 0.9%w/v). The amount of Zidovudine released from the gel was determined by spectrometric method. The results show a slower release of Zidovudine from liposomes incorporated in Carbopol940 and HPMC K4 100 gel.

Table-1 : Composition, Hydration time, Mean particle size

S. No.	PC: CH (mg)	Drug (mg)	Hydration time	EE
1	200:200	10	02	35±0.8
2	200:100	10	04	42±1.2
3	200:80	10	06	53±1.0
4	200:80	10	08	59±1.6
5	200:60	10	08	51±1.8

PC- Phosphotidyl Choline

CH-Cholesterol

EE-Entrapment Efficiency

(n=3)

Table-2 : Percentage release of conventional liposomes and Carbopol940 gel 2%, 3%

S.No.	Time in hours	% Release from conventional liposomes	% Release from Carbopol 940 2%	% Release from Carbopol 940 3%
1	0	0	0	0
2	0.5	12.5	8.64	7.54
3	1	21.45	12.56	10.65
4	1.5	29.56	16.52	13.85
5	2	38.2	22.5	18.45
6	2.5	50.74	25.64	23.4
7	3	62.48	27.01	25.87
8	3.5	71.25	29.47	28.63
9	4	84.6	31.25	30.57
10	6	98.01	42.52	39
11	8	98.23	51.96	44
12	10	98.56	63.78	52
13	12	98.99	69.35	61
14	14	98.74	76.89	66.7
15	16	98.01	82.45	73.3
16	18	99.01	90.12	80
17	20	98.38	98.56	88
18	22	98.12	98.23	93.4
19	24	98.64	98.47	94.78

(Mean±SD, n=3)

Figure-2 : Percentage release of conventional liposomes and Carbopol940 gel 2%, 3%

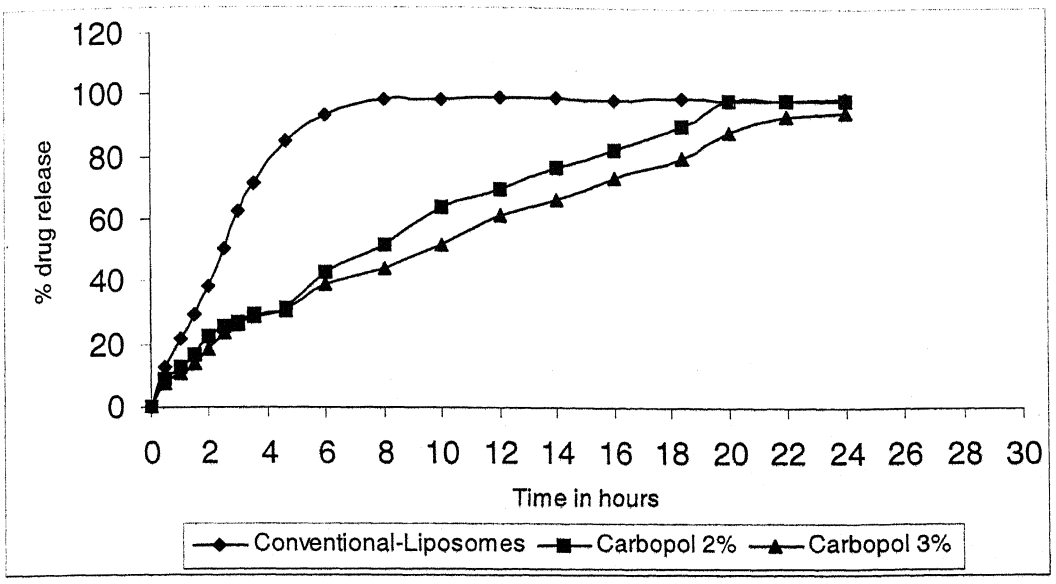
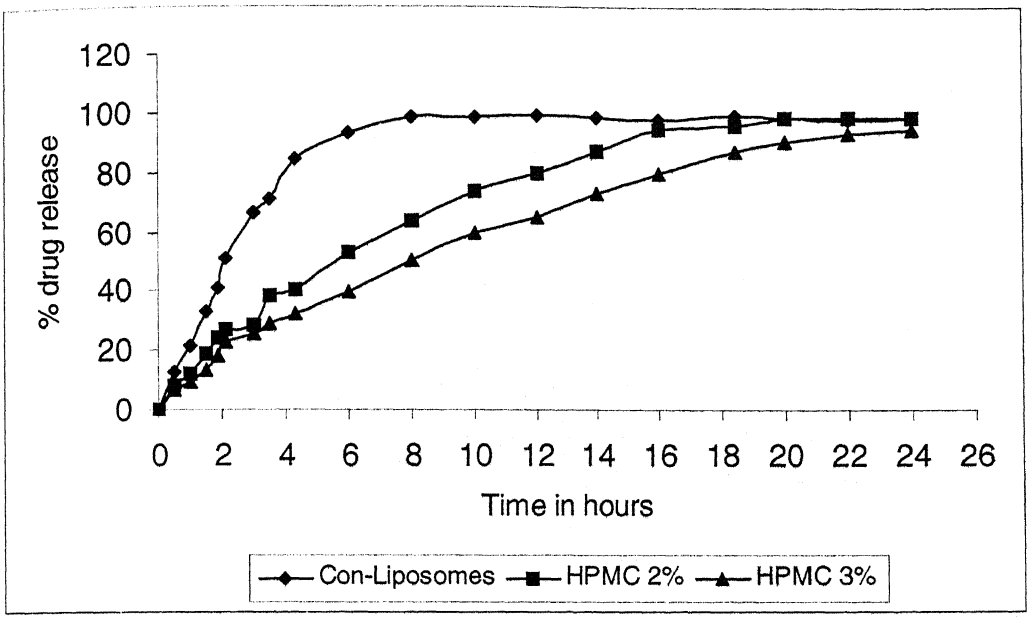


Table-2 : Percentage release of conventional liposomes and HPMC K4 100 gel 2%, 3%

S.No.	Time in hours	% Release from conventional liposomes	% Release from Carbopol 940 2%	% Release from Carbopol 940 3%
1.	0	0	0	0
2.	0.5	12.5	7.99	6.58
3.	1	21.45	12.35	9.65
4.	1.5	32.6	18.54	13.2
5.	2	40.6	23.86	17.99
6.	2.5	50.74	26.53	22.56
7.	3	66.2	28.34	25.62
8.	3.5	71.25	38.1	28.65
9.	4	84.6	40.25	32.47
10.	6	98.01	53.2	39.65
11.	8	98.23	64	50.14
12.	10	98.56	74	59.6
13.	12	98.99	80	65.2
14.	14	98.74	87.4	73.1
15.	16	98.01	94.6	79.5
16.	18	99.01	96	87.4
17.	20	98.38	98.56	90.6
18.	22	98.12	98.23	93.4
19.	24	98.64	98.47	94.78

(Mean±SD, n=3)

Figure-2 : Percentage release of conventional liposomes and HPMC K4 100 gel 2%, 3%



Invivo animal studies to be closer to the application of liposomes in humans. An appropriate viscosity of liposomal preparation is required. Carbopol940 2% and HPMC K4 100 2%, showed slower release than Carbopol940 1% and HPMC K4 100 1%, so we took Carbopol940 2% and HPMC K4 100 2% for the animal Invivo studies. The initial release was 38% with period of 8 hours and after it maintained constant around at 44%.

Table-3 : Invivo percentage release of HPMC K4 100 2% and Carbopol 940 2%

S.No.	Time in hours	Cumulative % Release of HPMC K4 100 2%	Cumulative % Release of Carbopol940 2%
1.	0	0	0
2.	0.5	6.52	5.63
3.	1	8.45	7.58
4.	1.5	11.23	9.98
5.	2	14.25	11.42
6.	2.5	21.54	16.58
7.	3	25.64	20.36
8.	3.5	28.65	25
9.	4	31.87	28.75
10.	6	33.5	29.21
11.	8	34.9	30.84
12.	10	35.6	31.66

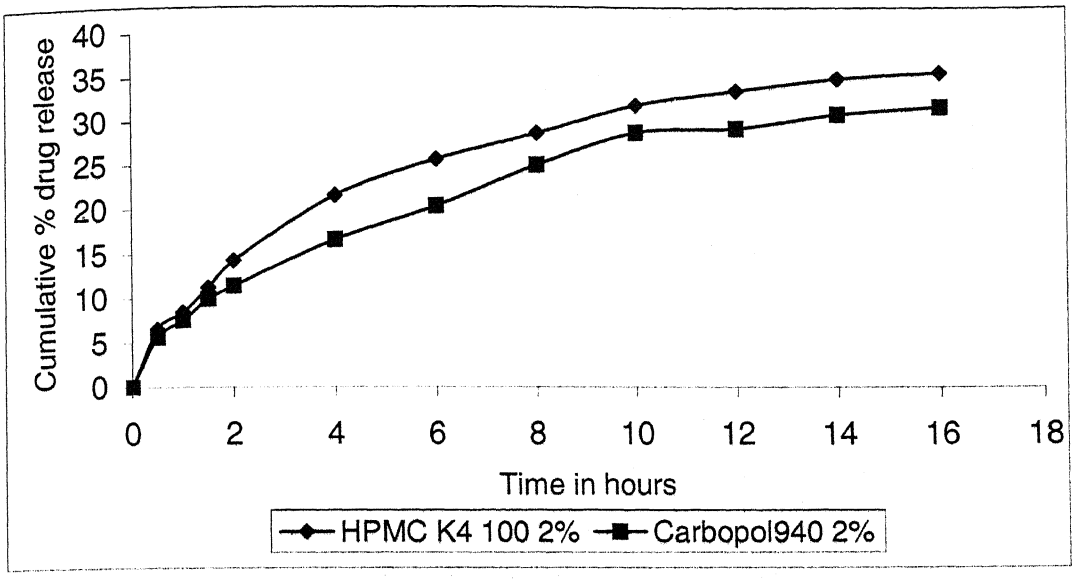


Figure-3: Invivo percentage release of HPMC K4 100 2% and Carbopol 940 2%

The slower release is due to the skin permeation of the drug because the drug has to cross several barriers in the skin. The prolong release can be achieved by their incorporation in vehicle suitable for topical self-administration. The prolonged retention on the skin is often required for the desired therapeutic effect; research efforts have been directed to using hydrophilic polymers with adhesive character to improve drug delivery via skin. It has already proven that liposomes are fairly compatible with polymers derived from cross-linked polymers. Therefore the research work confirmed to choose a gel made of Carbopol 940 and HPMC K4 100 polymers as a vehicle for liposomes.

CONCLUSION:

Topical formulations containing Zidovudine-loaded liposomes embedded in to the structured vehicles of Carbopol940, HPMC K4 100 have been prepared and evaluated. The release rates of Zidovudine from the topical gels were affected in the invivo and invitro studies. Comparing the invitro and invivo liposomal gels, the release rate of liposomes entrapped drug was prolonged the release rate of drug. The topical delivery of drug through liposomal gel showed the suitability of delivery systems for prolonged action of topically applied of hydrophilic drugs.

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Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope

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Effects of pH, Salt, Temperature on conventional liposomes size enlargement analyzed by optical microscope

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Institute of Pharmacy, Bundelkhand University, Jhansi-284003

ABSTRACT:

The objective of the present study was to investigate the effects of experimental conditions like pH, salt, temperature on the interactions of conventional liposomes. The model drug Zidovudine was entrapped in the liposomes composed of Phosphotidyl Choline and cholesterol in the ratio of 200mg: 80mg and prepared by thin film hydration method. Liposomes preparation was characterized and compared for particle size, mean dispersion, entrapment efficiency, DSC, Zeta potential and the size enlargement studies were carried out in optical microscope by using Magnus.

Key words: Liposomes, zidovudine, temperature, size enlargement, pH, salt.

INTRODUCTION:

Liposomes are becoming increasingly important as carriers of biologically important molecules in living system. (**Tyrell *et al.*, 1976; Papahadjopoulos, 1978; Gregoriadis, 1980; Roerdink *et al.*, 1981**). Liposomes have a variety of routes of administrations including oral, dermal, intravenous, ophthalmic (**Labris et al, 2003**).Liposomes mimic cells and are used for encapsulation and sustained release of drugs in modern therapies. They are aggregates containing a continuous bilayer of phospholipids around in aqueous space. There has been much interest in liposomes as drug delivery carriers, not only because of wide range of biologically active substances can be encapsulated,but that can be administered to man or animals with out any adverse effect. Incorporation in to liposomes can reduce the toxicity and produce sustained release (**Genin et al**). Liposomes, after three decades of research, are still gaining increasing interest with special emphasis more recently on their use as drug carrier systems. For therapeutic purpose they must be loaded with active substances. This is more achieved with lipophilic and hydrophilic molecules as they have tendency to be incorporated in the liposomal membrane. This is more essential achieved with amphiphile molecules like phospholipids and cholesterol as they have a tendency to be incorporated in the liposomal membrane. In contrast, hydrophilic molecules must be encapsulated in the aqueous interior, which in general, cannot easily be performed in an efficient manner (**M.J.Ostro1987**). A variety of liposomes preparations were introduced and they have to fulfill the basic requirements (a) Produced liposomes should be homogeneous (b) Efficient of

encapsulation of hydrophilic drugs. Where as thin film hydration technique achieve high efficiency and easy method to produce in the laboratories (**R.L. Juliano et al**). The concept of this project was fairly to entrap hydrophilic drugs like zidovudine. The ratio of volume inside the liposomes compared to the total aqueous volume of the preparation is encapsulation efficiency. The increasing lipid concentration more liposomes per unit volume of the preparation are formed. When phospholipid dispersed in aqueous medium at certain concentrations results in highly viscous dispersions up to semisolid consistency. So, the phospholipid, cholesterol and hydration times were changed to achieve maximum entrapment efficiency. The prepared liposomes were investigated at different temperature, pH and salt concentration for their stability.

Zidovudine, the first anti HIV compound approved for clinical use is widely used for the treatment of AIDS either alone or combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of zidovudine its dose dependant hematological toxicity, low therapeutic index, short biological half life and poor bioavailability (**K.D.Kieburtz et al**). Zidovudine available in the market as conventional tablet form. After oral administration, it is rapidly absorbed from GIT exhibiting a peak plasma concentration of 1.2µg/ml at0.8 hours (**R.W.Klecker et al**). In the systemic circulation, it is first converted to Azidothimidine triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of zidovudine is 4 hours.

MATERIALS AND METHODS:

Materials:

The materials used in the study were Zidovudine a gifted sample of Alkem Laboratories Ltd, Raigad. Phosphotidyl choline, Qualigens, Mumbai. Cholesterol, Qualigens, Mumbai. Chloroform, Merck, Mumbai. Methanols, Qualigens, Mumbai.were Purchased. All in the study, other ingredients used were of analytical grade.

Methods:

Preparation of liposomes:

Multilammellar liposomes were prepared by using thin film hydration method (**A.D.Bangham et al**)) Accurately weighed quantity of Drug, Phosphotidyl Choline, Cholesterol was taken. Phosphotidyl Choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flask (**G.Gregoriadis et al**). The chloroform and methanol mixture was evaporated at 45°C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing 0.9%w/v Saline solution for 8 hours until vesiculation was completed.

Estimation of Entrapped Drug in Liposomes

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to configuration in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15 000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained (**Kulkarni et al**). The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant.

Transmission electron microscopy:

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

Determination of zeta potential:

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential (**D.J.Crommelin et al**).

RESULTS AND DISCUSSION:

Liposomes have been used as a carrier system to deliver medications in to the skin in order to achieve the therapeutic effect with lower systemic absorption. Several novel carrier systems were suggested to be appropriate for topical drug delivery of liposomes. To achieve the desirable therapeutic affect of liposomes as drug carriers, they must be loaded with sufficient amount of active compounds. Therefore liposomes with Zidovudine were prepared by thin film hydration method with the effects of the formulation variables, lipid phase composition (Phosphotidyl Choline and cholesterol) and hydration time on the drug entrapment efficiency, vesicle size and mean distribution of liposomes. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). Regardless of the preparation procedure used, liposomes were of a mean diameter around 1110.509nm in diameter with the low cholesterol level. In the optical microscope it was found that the average diameter of the liposome vesicle was 2.467μm. The mean particle size and charge of the liposome were increased with the increased concentration of cholesterol. How ever, liposome prepared by the thin film hydration method was of a more homogenous size distribution. This was probably a consequence of the preparation procedure, in the better case.

Table-1: Composition, Hydration time, Mean particle size

S. No	PC: CH (mg)	Drug (mg)	Hydration time	EE
1	200:200	10	02	35±0.8
2	200:100	10	04	42±1.2
3	200:80	10	06	53±1.0
4	200:80	10	08	59±1.6
5	200:60	10	08	51±1.8

PC- Phosphotidyl Choline
CH-Cholesterol
EE-Entrapment Efficiency
(n=3).

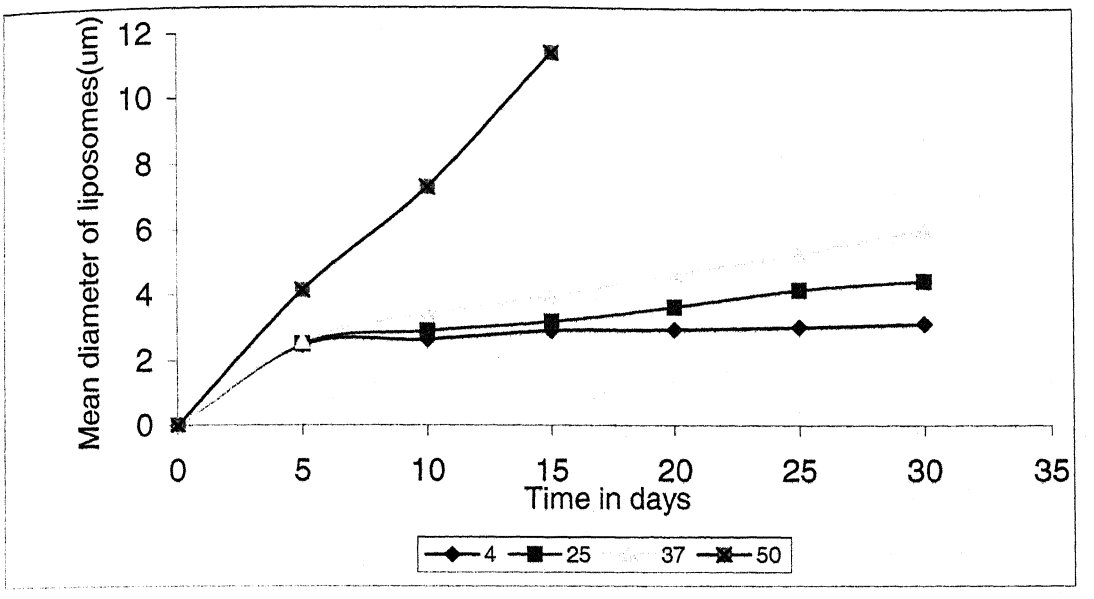
Effect of temperature of liposomes size enlargement:

The conventional liposomes containing zidovudine were taken and stored at different temperatures like 4°C, 25°C, 37°C, and 50°C for 30 days. The influences of temperature on liposomes diameter as a function of time have been investigated and are shown in the fig-1 and values of the size enlargement of liposomes shown in table–2. In the higher temperatures, the liposomes may aggregates and form clusters. This investigation is to find out the effects on size enlargement of liposomes and its suitable storage temperature. The liposomal coalescence is not reversible and it is sensitive to temperature**(B.L.Gamon et al)**. The aggregation of liposomes due to Brownian motion while increasing the temperature, there will be increasing in motion of the liposomes vesicles. The reaction limited clusters aggregation process, the average cluster size grows exponentially with the time. After aggregation liposomes tends to form large vesicles via coalescence. In this case that the decreasing the temperature of zidovudine liposomes increased the viscosity of liposomal suspension and the liposomal coalescence rate decreased. So it is shown in this study that a low temperature i.e. 4°C increases the stability of liposomes in relation to their size.

Table-2 Effect of temperature on liposome size enlargement

S.No	Time in days	4°C	25°C	37°C	50°C
0	0	0	0	0	0
1	5	2.458	2.498	2.575	4.13
2	10	2.635	2.891	3.4	7.3
3	15	2.896	3.17	3.94	11.4
4	20	2.941	3.621	4.61	-
5	25	3.01	4.15	5.28	-
6	50	3.126	4.426	6	-

Figure-1 : Effect of temperature on liposome size enlargement



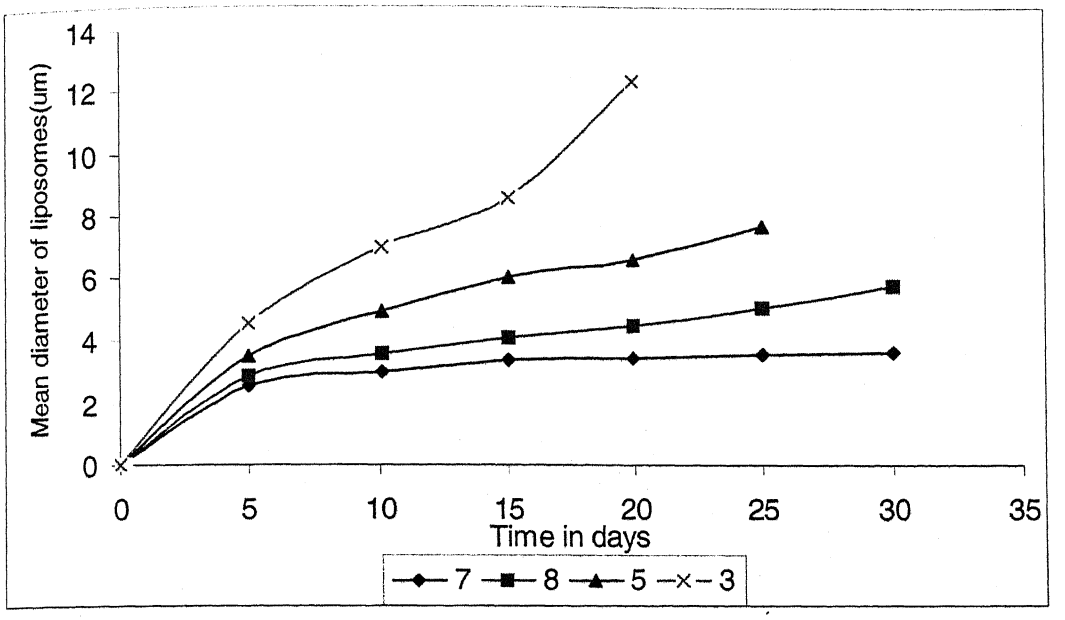
Effect of pH on liposomes size enlargement:

The conventional liposomes containing zidovudine were stored at different values like 3,5,7,8. The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-3. It was found that the effect of pH on liposome stability is significant **(D.D.Lasic et al).** The pH range above5-8, there is no significant change in the size enlargement. But the decreased pH (pH-3), the rate of membrane fusion may occur in the neutral liposomes.

Table-3: Effect of different pH on liposomes size enlargement

S.No	Time in days	Effect of size in pH-7	Effect of size in pH-8	Effect of size in pH-5	Effect of size in pH-3
0	0	0	0	0	0
1	5	2.54	2.88	3.53	4.52
2	10	2.98	3.6	4.95	6.98
3	15	3.37	4.07	6	8.56
4	20	3.47	4.46	6.56	12.34
5	25	3.6	5.06	7.7	--
6	30	3.67	5.75	--	--

Figure-3 : Effect of different pH on liposomes size enlargement



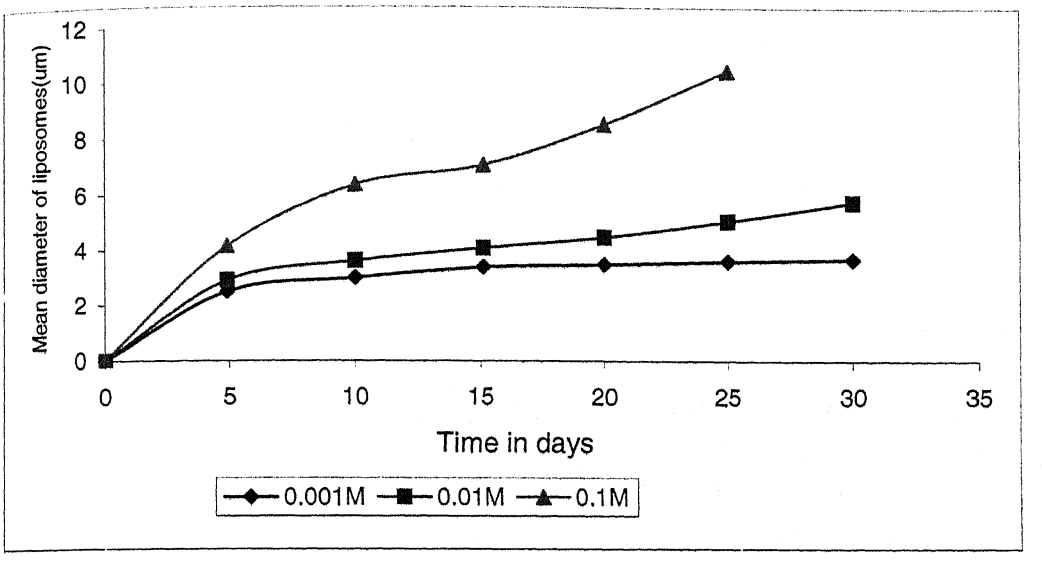
Effect of calcium chloride on liposomes size enlargement:

The conventional liposomes containing zidovudine were stored at different molar ratio of calcium chloride concentration. I.e. 0.001M, 0.01M, 0.1M solution at 25°C at pH7 reported (**M. Juan Ruso et al**). The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-4.in the range of 0.001M to 0.01M there is no significant change in the size enlargement. Above 0.01M i.e. 0.1M calcium chloride salt may induce aggregation behavior of conventional liposomes occurs only at a high concentration salt level.

Table-4 Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement

S.No	Time in days	0.001M CaCl2	0.01M $CaCl_2$	0.1M $CaCl_2$
0	0	0	0	0
1	5	2.48	2.88	4.13
2	10	2.98	3.6	6.35
3	15	3.37	4.07	7.05
4	20	3.47	4.46	8.52
5	25	3.6	5.06	10.45
6	30	3.67	5.75	--

Table-4 : Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement



CONCLUSION

The stability of liposomes with respect to aggregation was evaluated by using different salt concentrations, different pH and different temperatures. With the increasing concentration of the electrolytes were more effective for liposome aggregation. The highest level of stability of liposomes was observed at pH-7. The temperature dramatically influenced the aggregation. Low temperature was suitable for the storage of liposomes.

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Natural Soya Lecithin As A Drug Delivery-A Review

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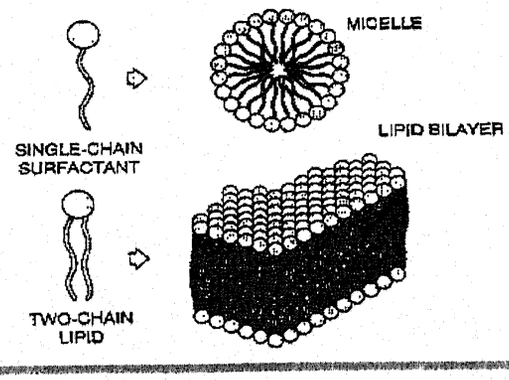
ABSTRACT

The evolution of the science and technology of liposomes as a drug carrier has passed through a number of distinct phases. Because they exhibit peculiar properties due to their structure, chemical composition amphiphile nature, physico-chemical characters and colloidal size, which are used in various applications. These properties point to several applications as the solubilizer for insoluble drugs, dispersants, and sustained release system, delivery system for the encapsulated substance, stabilizer, protective agents, and micro reactive being the most obvious ones. Yet interest in liposomes, especially among academic workers, spread rapidly we attribute this to the remarkable structural versatility of the system, which enables the design of countless liposomes versions to satisfy particular needs in terms of both technology and optimal function in vivo.

INTRODUCTION

Liposomal vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes (**Abraham W. et al.**). Liposomes were discovered in the mid of 1960' s and originally studied as cell membrane model Paul Ehrlich coined the term "magic bullet" in 20th century where carrier system's was proposed to simply carry the drug to its of action and releasing its selectively while non target sites should absolutely be exempted from drug effect (**Crommelin O.J.A. et al.**). The exploration and progressive advent of liposomal drug delivery system has rekindled interest in magic bullet approach, for surely man's ingenuity can find means for directing these drugs filled packed or lipid bilayer vesicles to specific cell or anatomical sites within the body (**Barenholz Y. et al.**). Liposomes were described as a model of cellular membranes and quickly were applied to the delivery of substances to cells. Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. When mixed in water under low shear conditions, the phospholipids arrange themselves in sheets, the molecules aligning side by side in like orientation, "heads" up and "tails" down. These sheets then join tails-to-tails to form a bilayer membrane, which encloses some of the water in a phospholipids sphere (**Suggy S. et al.**).



Mechanism of liposomal formation

Liposomes are formed open hydration of lipid molecules normally lipids are hydrated from a dry state (thin or thick lipid film, spray dried powder), and stacks of crystalline bilayers become fluid and swell myelin-long, thin cylinders grow and upon agitation detach self close in to large, multilameller liposomes because this eliminates unfavorable interactions at the edges. Once the large particles are formed they can be either broken by mechanical treatment in to smaller bilayered fragments, which close into smaller liposomes (**Lasic D. D. et al.**).

Classification of liposomes

Multilameller vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated from one another by a layer of aqueous solution. These vesicles are over several hundred nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50 nm (according to some authors up to 100 nm) in diameter. Large unilamellar vesicles (LUV) are, in fact, a very heterogeneous group of vesicles that, like the SUVs, are surrounded by a single lipid layer. The diameter of these liposomes is very broad, from 100 nm up to cell size (giant vesicles) (**Baiydia et al**) Besides the technique used for their formation the lipid composition of liposomes is also, in most cases, very important (**Allen T. M. et al.; Swarbrick J. et al.**).

Methods of liposomes preparation

Basic studies on liposomal vesicles resulted in numerous methods of their preparation and characterization (**Storm G. et al.; Vyas .S P.et al.**).

A. Hydration stage

1. Mechanical methods
   - Vortexing or hand shaking of phospholipid dispersions (MLV)
   - 'Microfluidizer' technique (mainly SUV)
   - High-shear homogenization (mainly SUV)
2. Methods based on replacement of organic solvent(s) by aqueous media
   - Removal of organic solvent(s) before hydration (MLV, OLV, SUV)
   - Reverse-phase evaporation (LUV, OLV, MLV)
   - Use of water immiscible solvents: ether and petroleum-ether infusion (solvent vaporization) (MLV, OLV, LUV)
   - Use of water miscible solvents such as ethanol injection (MLV, OLV, SUV)
3. Methods based on detergent removal
   - Gel exclusion chromatography (SUV)
   - 'Slow' dialysis (LUV, OLV, MLV)
   - Fast dilution (LUV, OLV)
4. Methods based on size transformation and fusion
   - Spontaneous fusion of SUV in the gel phase (LUV)

- Freeze-thawing (MLV)
- Freeze-drying (MLV)

B. Sizing stage

1. High pressure extrusion
2. ***Low pressure extrusion***
3. Ultrasonic treatment

C. Removal of non-encapsulated material

1. Dialysis
2. Ultracentrifugation
3. Gel-permeation chromatography
4. Ion-exchange resins

Targeting via Liposomes

Depending on the need, one can use SUV type or MLV type vesicles for effective entrapment and delivery of the drug to the target tissues or cells (**Baidya S. et al.**). Nevertheless, charge properties and interactions of the active compound with vesicle forming molecules will determine the effect of entrapment, i.e., the amount of the compound that can be "loaded" into a single vesicle (**Gabizon A. et al.**). On the other hand, the composition of the molecules used for the formation of the vesicular structure will, at least, affect the fate of vesicles from the site of their introduction as well as the interaction with component of the body (e.g., surface charge, serum proteins, lipoproteins, opsonin system, phagocytic system and finally target cells. In the earlier studies, when therapeutically active substances were not easily available, most of the experiments were done using a marker compound (**Bandak S. et al.**). The results, however, were not the same as those obtained in experiments in which an active substance was used and the conditions were more related to the real situation (ex-vivo, in-vivo). These findings implicate the necessity for studies in which an active substance is used and the conditions of the experiments resemble, as closely as possible, those of therapeutic Liposomal (vesicular) drug application. The benefits of liposomal formulations were already demonstrated clinically and stimulate many laboratories (research and pharmaceutical) in their efforts to introduce new Liposomal vesicular drugs (**Lasic DD. et al.**).

Liposomes are well established as drug carriers in topical treatment of diseases, especially in dermatology. They can enhance penetration of encapsulated hydrophilic drugs into the skin to enable a proper therapeutic effect. Because of they are able to carry with them any enclosed substances into the dermis and to 'the individual cells (**Zhang YP et al.**).

Sustained release of the incorporated drug,

Liposomes are typical vehicles, which are able to transport dermatological and cosmetic active agents of different types. The active agents are encapsulated und protected against environmental influences. Liposomes spread out excellently in the horny layer of the skin and form depots of active agents. Aqueous dispersions show a clear to milky appearance

according to the size of the Liposomes. Similar to the horny layer of human skin, liposomes consist of one or several bilayers of phosphatidylcholine. Liposomes without active agents ("empty liposomes") show all dermatological and cosmetic effects of phosphatidylcholine (**Zuidam NJ. et al.; Vernooij EAAM et al.**).

The size of these spheres is very small, in the order of a nanometer. As illustrated, the spheres are hollow inside and enclose some of the liquid material in which they were formed (inclusion). Because of the small size of the phospholipid molecule and microspheres, they can pass through the epidermis and act as a carrier for the enclosed substances.

Release Kinetics of Liposomal Payload

Liposomes are most useful for being able to transfer and deliver active ingredients to the application site of formulation. The liposome wall is very similar, physiologically, to the material of cell membranes. Application of formulation over skin area causes deposition of liposomes on the skin and begins to merge with the cellular membranes. In the process, the liposomes release their payload of active materials into the cells. As a consequence, not only is delivery of the actives very specific directly into the intended cells but also the delivery takes place over a longer period of time. Liposomes exhibit better stability, penetration and efficacy at lower usage levels (**Lasic DD. et al.**).

Liposomes as a delivery system can be made to release their payload under a variety of conditions.

- Slow / Fast Release of Hydrophilic Payload
- Slow / Fast Release of Hydrophobic Payload
- Bilayer Composition
    1. Chain Length
    2. Saturation
    3. Lipid Class
- Physical Configuration of Liposome
- Solvent-Dependent Release
- pH-Dependent Release
- Temperature-Dependent Release

Advantages of Liposomes

The characteristics of liposomes also yield a variety of other formulation benefits (Daan J. A. et al.).

- Controlled Delivery System
- Biodegradable, Non-Toxic
- Carry Both Water and Oil Soluble Payloads

- Can Solubilize Recalcitrant Compounds
- Prevention of Oxidation
- Protein Stabilization
- Controlled Hydration

Applicability of Liposomes

One may conclude that, at present, the term "liposomes" covers not only phospholipid based vesicles but also other vesicular structures with properties identical or similar to those of classical, natural phospholipid based Liposomes. In the early 70's the use of liposomes as a drug carrier system was proposed by Gregoriadis & Ryman. Since this first report, liposomes were developed as an advanced drug delivery vehicle. They are generally considered non-toxic, biodegradable and non-immunogenic (Osborne D. W. et al.). Associating a drug with liposomes markedly changes its pharmacokinetics and lowers systemic toxicity; furthermore, the drug is prevented from early degradation and/or inactivation after introduction to the target organism (Gabizon A. et al.). The use of liposomes or, in general, vesicular structures for the delivery of various active compounds is recognized in relation to water solubility of the compound. When the compound is water soluble, the size and volume of the aqueous compartment of the vesicle is crucial (Daan J. A. et al.). In contrast, hydrophobic compounds will prefer incorporation into the lipid (amphiphile) layer that constructs the vesicle. In such a case, the size of the aqueous compartment is not important.

Therapeutic applications of liposomes

Liposomes are used for the following range of therapeutic and pharmaceutical applications (**Vyas .S P. et al.**):

1. Liposomes as drug protein delivery vehicles.
   - Controlled and sustained drug release *in situ.*
   - Enhanced drug solubilization.
   - Altered pharmacokinetics and biodistribution.
   - Enzyme replacement therapy and lysosomal storage disorders
2. Liposomes in antimicrobial and antifungal (lung therapeutics) and antiviral (anti-HIV) therapy
   - Liposomal drugs
   - Liposomal biological response modifiers
3. Liposomes in tumour therapy
   - Carrier of small cytotoxic molecules
   - Vehicle for macromolecules as cytokines or genes

4. Liposomes in gene delivery.
   - Gene and antisense therapy
   - Genetic (DNA) vaccination
5. Liposome Immunology.
   - Immunoadjuvant
   - Immunomodulator
   - Immunodiagnosis
6. Liposomes as artificial blood surrogates.
7. Liposomes as Radiopharmaceutical and Radiodiagnostic carriers
8. Liposomes in cosmetics and dermatology
9. Liposomes in enzyme immobilization and bioreactor technology.

CONCLUSION

Liposomes have been realized as extremely useful carrier systems, additive(s) and tools in various scientific domains. Thus, liposomes over the years have been investigated as the major drug delivery systems due to their flexibility to be tailored for varied desirable purposes. The flexibility in their behavior can be exploited for the drug delivery through any route of administration and for-any drug or material irrespective of its physicochemical properties (Swarbrick J. et al.). The uses of liposomes in the delivery of drugs and genes to tumour sites are promising and may serve as a handle for focus of future **research (Storm G. et al.).**

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